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FAMILY-SIZE IN TITMICE OF THE GENUS *PARUS*

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INTRODUCTION

In previous papers the hypothesis was advanced that, in birds, family-size is ultimately limited by natural selection, and that the normal clutch-size corresponds to the maximum number of young which can be successfully raised to maturity. It was shown that, as brood-size increases, so does the proportionate mortality among the young, either in the nest as in *Apus melba* (Lack and Arn, 1947), or shortly after the young leave the nest, as in *Sturnus vulgaris* (Lack, 1948). In the above two species, the increased mortality was sufficient to offset the initially higher broodsize, but it was not sufficient to do so in two species of *Turdus*, in which the position is complicated by the existence of phenotypic and adaptive modifications in clutch-size (Lack, 1949). Knowledge of these papers is assumed, and references in the present paper to the above species are cited from them.

In Western Europe, there are six common species of *Parus;* the Great Tit *P. major,* Blue Tit *P. caeruleus,* Coal Tit *P. ater,* Marsh Tit *P. palustris,* Willow Tit (Chickadee) *P. atricapillus* and Crested Tit *P. cristatus.* Each of these has a somewhat different clutch-size. Moreover, in each species the average clutch-size differs somewhat in different regions, in different localities in the same region, in different months of the year, and in different years. In the Great Tit, *P. major,* for instance, the average clutch-size may, under different circumstances, be as small as 4 or as high as 12 eggs, while clutches of between 3 and 15 eggs are not uncommon, and the extremes are even greater. Hence the reproductive rate varies greatly, and since the number of potential offspring is correspondingly affected, natural selection is presumably exerting a powerful influence.

As most European species of titmice are abundant and breed freely in nesting boxes, nesting records are easy to collect, and the present data are perhaps the most extensive so far available for the variations in reproductive rate of any animal in the wild. The main sources used are (i) nesting data obtained by the writer with the help of the Forestry Commission of Great Britain 1947–49, to be published in detail after 1950; (ii) nesting data from the Institut van den Plantenziektenkundigen Dienst, Wageningen, Holland, 1922–36, kindly loaned by Dr. H. N. Kluijver, who is himself

publishing a biological study of *P. major;* (iii) clutch records from Sweden collected by S. Durango; (*iv*) records of *P. major* banded and later recovered by Dr. H. N. Kluijver at Wageningen, by the Hon. G. Charteris in England and by the Vogelwarte Sempach (per Dr. Schifferli) in Switzerland. The above data are unpublished, and I am deeply indebted to the persons named for making them available to me. A copy of the raw data, with fuller analyses than those published here, and details of the statistical tests made for this paper, have been deposited at the Edward Grey Institute, Oxford.

Nesting Success (Omitting Total Losses)

In all the species studied so far, hatching success was similar for clutches of different size. Table 1 shows that this also applies to *P. major* and *P. caeruleus* in England, and it is presumably general.

In all the passerine species studied previously, nestling survival was also similar for broods of different size. The data for first broods of titmice in England in 1947–49, set out in table 2, show that nestling survival was high, and that it did not vary significantly with brood-size. However in one area in 1949, mentioned in a footnote, there was an unusually high mortality among the young, due to starvation from unknown causes. Here the mortality was perhaps higher in large than small broods, but the data were not extensive enough for a firm conclusion.

These English data can be amplified by far fuller data for four species of *Parus* in Holland, set out in table 3. As clutch-size declines as the season progresses (see later), the records have been analyzed separately for different times of the year, the date given being that on which the first egg was laid. All clutches laid April 1–29, and some of the later ones, are first broods. There then follow repeat clutches, laid after the destruction of first attempts, and then some genuine second broods (which are rare in England). In Holland, each observer recorded only the number of eggs laid in each nest and the number of young alive on his last visit, hence it is not possible to analyze egg and nestling losses separately, as it was for England.

Table 3 shows that there is a definite,

Table 1. *Hatching success of first broods in England (1947–49)*

Clutch-size	Number of eggs		Per cent hatched
	Laid	Hatched	
Parus major			
6–9	458	419	91
10–12	1,123	1,051	94
13–15, 18	375	345	92
Parus caeruleus			
6–9	259	240	93
10–12	1,007	935	93
13–15	605	577	96
16–19	86	80	93

Table 2. *Nestling survival of first broods in England (1947–49)*

Initial brood-size	Number of young		Per cent flying
	Hatched	Flying	
Parus major			
2–6	101	96	95
7–9	588	584	99
10–12	812	796	98
13–15	215	215	100
Parus caeruleus			
1–6	17	14	(82)
7–9	313	305	98
10–12	834	820	98
13–15	508	495	97
16–18	51	48	94

Note: In one area of planted conifers in 1949, nestling survival was much lower, 60% of 407 hatched in *P. major* and 49% of 198 hatched in *P. caeruleus*; possibly survival was slightly higher in smaller than larger broods, but the difference was not significant.

TABLE 3. Over-all nesting success in Holland.

Clutch-size	Eggs laid	Young flying	Per cent flying	Eggs laid	Young flying	Per cent flying	Eggs laid	Young flying	Per cent flying	Eggs laid	Young flying	Per cent flying
A. *Parus major*												
	April 1–29			April 30–May 9			May 10–29			May 30 on		
1–6	1,464	1,218	83	949	786	83	961	806	84	3,143	2,717	86
7–9	18,494	15,375	83	8,618	7,451	87	6,470	5,442	84	14,104	11,845	84
10–12	24,275	19,786	81	9,985	8,388	84	4,713	3,919	83	5,373	4,357	81
13–15	2,373	1,878	79	711	545	77	267	203	76	200	135	68
16–21	109	69	63	85	61	72	32	16	50	—	—	—
B. Other species—April 1–29												
	P. caeruleus			*P. ater*			*P. cristatus*					
1–6	404	305	76	887	669	75	1,385	1,180	85			
7–9	4,859	4,124	85	11,384	9,745	86	5,204	4,454	86			
10–12	17,352	14,687	85	7,175	6,034	84	280	240	86			
13–15	6,260	5,223	83	109	62	57	—	—	—			
16–21, 24	557	400	72	—	—	—	—	—	—			
C. Other species—Late nests												
	P. caeruleus (May 30–)						*P. ater* (May 20–)					
1–6	744	606	82				991	877	88			
7–9	2,291	1,848	81				7,614	6,697	88			
10–13	451	379	84				2,080	1,751	84			

though rather small, decrease in average nesting success as family-size increases, occurring in all four species of titmice at most seasons of the year. The decreases in question are statistically significant. The data do not reveal whether the differential mortality affected the eggs or the nestlings, but presumably it was the young birds which were involved. Titmice therefore differ from the other passerine species so far studied, which do not show a differential mortality with respect to brood-size before the time at which the young leave the nest. In *Apus melba*, however, there is a much higher differential mortality in relation to brood-size than there is in titmice.

The slightly higher mortality for broods of extremely small size, found in some of the Dutch data, has not been explained, but such small broods are often abnormal in various ways. That over-all success was lower in Holland than England probably means that 1947, 1948 and 1949 were unusually favorable years in England. It should be added that the total losses were omitted from these analyses. Total losses are due to predation (mainly by small boys or squirrels) or desertion (caused mainly by human disturbance), and such losses do not vary with brood-size.

SURVIVAL AFTER LEAVING THE NEST

In *Sturnus vulgaris* and *Turdus merula*, there was a proportionately heavier mortality among the young from broods of larger than smaller size, occurring shortly after the young left the nest. This differential mortality was assessed indirectly, by analysing the proportions of banded birds from each brood-size which were recovered after they were at least

three months old. A similar analysis has been made in table 4 for *P. major* in one area in Holland, in one area in England, and for the whole of Switzerland. The high rates of recovery in Holland and England were due to the fact that the banders in question also trapped many titmice in winter. Some but not all Swiss banders did the same, but as average brood-size varies regionally in Switzerland (see later), the inclusion of data from trapped birds badly biasses the results.

TABLE 4. *Recoveries of* Parus major *after leaving nest, of all young at least 3 months old*

Brood-size	No. of young banded	No. of young recovered	Per cent recovered
A. Dutch broods laid April 1–May 9 (H. N. Kluijver)			
1–3	79	6	7.6
4–6	677	59	8.7
7–9	1,592	118	7.4
10–13	704	49	7.0
B. Dutch broods laid after May 9 (H. N. Kluijver)			
1–6	569	28	4.9
7–11	612	28	4.6
C. British first broods (Hon. G. Charteris)			
2–7	225	22	9.8
8–13	310	20	6.5
D. Swiss broods banded before June 16 (all workers)			
2–6	3,714	14	0.38
7–9	7,791	20	0.26
10–15	3,287	6	0.18
E. Swiss broods banded after June 16 (all workers)			
2–6	1,059	6	0.57
7–11	958	2	0.21

Swiss recoveries of trapped birds: In 1st broods: 56 from broods of 2–6, 118 from broods of 7–9 and 44 from broods of 10–15; in 2nd broods: 18 from broods of 2–6, and 14 from broods of 7–11.

For instance, the most active trapper worked in an area where brood-size was unusually high. The totals of trapped birds in Switzerland are shown as a footnote to table 4.

The data in table 4 suggest the possibility that in *P. major,* as in other passerine birds, there may be a rather lower survival from broods of large than broods of small size. The differences are not, however, statistically significant, nor are they nearly so large as in the other species studied. In particular, they are not nearly large enough to offset the advantage of starting with an initially larger brood.

Gibb (1950) has shown that for *P. major* each nestling in a brood of large size receives on the average rather fewer feeding visits per day, and weighs on the average rather less, than each nestling in a brood of small size. The differences, though statistically significant, are not large, but they were obtained in two summers with excellent weather when food was very abundant. The differences might be expected to be greater in a year when food is harder to obtain. This work supports the view that the chances of survival after leaving the nest might be rather lower for young titmice from broods of large than small size, but also that the differences in question would not be great enough to offset the initial advantage of a larger brood-size.

If, in titmice, families of larger size are not proportionately handicapped, the problem arises of why any individuals have families of smaller size. The same problem arose previously in connection with *Turdus ericetorum,* and it was then suggested that many of the clutch-size differences found in nature might be caused by environmental differences, and might have adaptive significance. The kinds of variation in clutch-size found among titmice in nature must therefore be investigated further.

Specific Differences

Each species of *Parus* has its characteristic clutch-size, which differs some-

TABLE 5. *Differences between first-brood clutches of same individual* P. major *in different years (Holland)*

Size of difference	0	1	2	3	4	5	6
Observed number	26	43	25	7	2	1	0
Expected number if varying independently	19	34	25	15	7	3	1
Obs.-Exp.	+7	+9	0	−8	−8		

$\chi^2 = 15.05$.

Note: The third line above gives the expected distribution on the assumption that the size of clutch laid by a bird in any year is independent of its clutch-size in the previous year. A comparison of the observed with the expected figures gives $\chi^2 = 15.05$ which is significant at 1%, thus disproving the hypothesis of independence of clutch-size.

what from that of other titmice species breeding in the same place at the same time of year. In Holland, for instance, the usual size of a first clutch of *P. cristatus* is 7 eggs, of *P. ater* 9 eggs, of *P. major* 9–10 eggs and of *P. caeruleus* 11–12 eggs. *P. caeruleus* and *P. major* normally breed side by side in the same broad-leaved woods, and *P. ater* and *P. cristatus* side by side in the same coniferous woods, while *P. major* also breeds in conifers, as, to a smaller extent, does *P. caeruleus*. Hence the differences concerned are not due primarily to differences in habitat. Nor are they due primarily to differences in breeding date, as they hold for clutches laid on the same date in the same year. It is therefore reasonable to infer that these specific differences have a hereditary basis.

INDIVIDUAL DIFFERENCES

That clutch-size has a hereditary basis is also suggested (but not proved) by the fact that each individual female tends to lay a clutch of relatively similar size in different years. H. N. Kluijver recorded the size of the first-brood clutch in successive years for various banded individuals of *P. major*. Using these data, the estimated variance of the difference between the first-brood clutches of all birds was 4.14 (73 degrees of freedom), while the estimated variance of the difference between clutches of the same individual was 1.27 (104 d.f.), the ratio 3.26 being highly significant for the degrees of freedom involved. The alternative test in table 5 also shows that the same individual tends to lay a clutch of similar size in different years. The same answer was obtained for the only two other species of birds in which this point has been studied, namely *Apus melba* and *Sturnus vulgaris*. In all three species, however, the clutch-size of each individual is not constant from year to year, and in *Parus* it seems particularly variable. Table 5 shows that in *P. major* it may vary by up to 5 eggs in successive first broods, and even greater differences are sometimes found between first and second broods of the same hen.

In *P. major*, it is possible that the tendency towards individual consistency in clutch-size is due not to heredity but to habitat, for there are local differences in average clutch-size (see later), and each female tends to stay for life in the same place. In *Apus melba* and *Sturnus vulgaris*, however, the possible influence of habitat can be ruled out, as the individual differences hold good for birds breeding in the same colony.

AGE

With the help of banded individuals, Kluijver (*in press*) has also shown that one-year-old individuals of *P. major* tend to lay slightly smaller clutches than older individuals. The average difference is a little below 0.5 egg, but seems to vary somewhat in different years. A rather smaller average clutch-size in the first breeding year was also found in *Sturnus vulgaris* and *Apus melba*, and I have suggested that it is a result of natural selec-

TABLE 6. *Seasonal and annual variations in clutch-size in English broadleaved woods*

Period	*P. major*		*P. caeruleus*	
	No.	Mean	No.	Mean
1947				
26–30 April	7	12.0	8	14.0
1–10 May	10	10.6	3	11.3
1948				
1–5 April	8	12.5	9	14.1
6–10 April	15	12.2	18	13.7
11–15 April	24	12.3	28	13.5
16–20 April	7	12.7	6	13.3
21–25 April	3	11.7	5	11.8
26–30 April	—	—	1	11.0
All May	13	8.2	4	8.3
1949				
15–18 April	—	—	26	11.8
19–20 April	19	11.0	49	11.6
21–23 April	52	10.3	65	11.3
24–25 April	34	9.6	24	9.9
26–30 April	26	9.2	25	9.3
1–5 May	6	8.3	10	8.9
6–10 May	—	—	7	7.7

tion, due to experienced parents being able, on the average, to raise slightly larger families than parents breeding for the first time.

SEASONAL VARIATIONS

The data set out in table 6 for England and in table 7 for Holland show that in titmice there is a marked decline in average clutch-size as the season progresses. This seems greatest in *P. caeruleus*, in which, in England in 1948, the average fell from 14 eggs in early April to 8 eggs in May, while in Holland the average over all years fell from 11.6 at the start to 6.3 at the end of the season. A similar but less marked trend is found in the other three species, and in all four species the differences are statistically significant. These variations occur whether the breeding season is early, as in 1948, normal as in 1949, or late as in 1947. Nor is the phenomenon due simply to the fact that repeat layings and second broods are smaller than first attempts. Considering only the true first broods, those laid in the first part of the season are on the average larger than those laid in the second half.

In *Turdus* spp., average clutch-size was found to be smaller in April than in May, but again smaller from June onwards. This agrees with the general trend found in passerine birds, and is attributable to the fact that in diurnal species the period available each day for collecting food is longer in midsummer than either earlier or later in the season. Hence, if other factors are equal, birds can on the average collect more food per day, and so raise larger families, in midsummer than either before or after (Lack, 1945).

Titmice constitute an exception to the

TABLE 7. *Seasonal variations in average clutch-size in Holland (1922–36)*

Period	*P. major*		*P. caeruleus*		*P. ater*		*P. cristatus*	
	No.	Mean	No.	Mean	No.	Mean	No.	Mean
Before March 31	—	—	—	—	10	7.1	31	7.26
Mar. 31–April 9	121	9.17	47	11.62	304	8.92	290	7.07
Apr. 10–19	1,454	9.43	714	11.27	977	8.86	420	7.11
Apr. 20–29	3,675	9.30	1,778	10.69	1,012	8.69	295	7.11
Apr. 30–May 9	2,288	9.07	916	10.05	466	8.20	107	6.70
May 10–19	737	8.66	288	8.77	247	8.30	68	6.57
May 20–29	633	8.47	184	8.09	578	8.45	104	6.25
May 30–June 8	1,265	8.21	334	7.53	655	7.95	97	6.10
June 9–18	1,123	7.72	227	7.07	341	7.42	37	6.34
June 19–28	349	7.11	55	6.85	82	7.06	8	—
June 29–July 8	71	6.58	10	6.3	36	6.50	2	—
After July 8	11	6.36						

Note: For all four species the seasonal differences are significant at the 1% level.

usual seasonal trend, as average clutch-size is smaller in May than in April. Presumably, therefore, some factor offsets the advantageous change in the daylength at this time of the year. Around Oxford, the breeding season of *P. major* and *P. caeruleus* is closely correlated with the brief period of abundance of defoliating geometrid caterpillars, notably *Cheimatobia brumata* (Gibb, 1950). After the caterpillars decline in numbers, which they do sharply in the second half of May, there is no other food available in the woods in anything approaching their abundance. This suggests that the decline in the clutch-size of titmice in May is an adaptation, correlated with the decline in the abundance of the food available for their young rather later. The connection is not, of course, direct, for there is a three-weeks interval between egg-laying and the time when the young titmice require feeding (one egg is laid each day and incubation takes 13–14 days; the young are another 18–20 days in the nest). Such "anticipatory" behavior is characteristic of the operation of natural selection. The duration of the caterpillar season in Holland is not on record, but that second broods of titmice are rare in England but frequent in Holland suggests that the period of abundant caterpillars may be more extended in Holland than in England.

Annual Differences

Titmice also show annual differences in clutch-size, i.e., there are, differences in the average size of first-brood clutches laid at the same place at the same time of year, but in different years. These are shown in Table 6 for English broad-leaved woods in 1948 compared with the same woods in 1949, and in table 8 for Holland as a whole ever the years 1922-1936. The annual differences in Holland are statistically significant for three of the four species studied, and are up to about 1.0 egg in size, which is, however, much smaller than the extent of the seasonal differences discussed in the previous section. Kluijver (*in press*) has shown for *P. major* that similar, indeed rather greater, annual differences in aver-

Table 8. *Annual differences in average clutch-size in Holland. For all clutches laid April 1–29*

Year	*P. major*		*P. caeruleus*		*P. ater*		*P. cristatus*	
	No.	Mean	No.	Mean	No.	Mean	No.	Mean
1922	34	9.06	25	11.40	18	9.00	57	7.42
3	284	9.38	125	11.13	44	8.55	85	6.91
4	104	10.11	35	11.23	35	9.06	45	7.07
5	486	9.69	152	11.20	86	8.92	133	6.74
6	301	10.17	115	11.46	122	9.28	84	7.00
7	320	9.22	131	10.73	216	8.37	51	6.86
8	275	10.10	140	11.20	315	8.61	58	7.02
9	100	9.80	34	10.35	161	8.52	68	7.21
1930	481	9.68	155	10.97	227	8.71	57	7.30
1	203	9.68	81	11.56	97	8.96	53	7.51
2	246	9.57	117	10.87	126	8.94	51	6.92
3	589	9.06	341	10.58	282	8.99	87	7.38
4	887	9.03	335	10.69	169	8.87	78	7.21
5	643	9.47	301	10.88	178	9.01	53	7.13
6	748	8.98	315	10.69	164	8.47	46	7.17

Notes: (i) For *P. major* and *P. ater* the differences are significant at 1% level, in *P. cristatus* at 5% level, and for *P. caeruleus* they are not significant; (ii) the correlations between the variations shown by the four species were all positive but were not statistically significant except (very barely) between *P. caeruleus* and *P. ater*.

age clutch-size are found when comparisons are restricted to one particular locality in different years.

For Holland as a whole, the annual variations in the four species of titmice showed positive correlations with each other, but these were not statistically significant except very barely in one case. For *P. major,* Kluijver (*in press*) found that the annual variations in four different localities in Holland were not correlated significantly with each other. Nor could he find correlations between the annual variations in clutch-size and the weather conditions in the different years. Annual differences in the average brood-size of *Turdus merula* were significantly correlated with those occurring in the related *T. ericetorum* in the same region. The situation in titmice is evidently more complicated than this, and requires further study. The significance of these annual differences is at present far from clear.

Local Differences

Titmice also show local variations in average clutch-size. The data from five localities in Holland in the first section of table 9 show that the average clutch-size differs in different places, by up to 3 eggs in *P. major,* and by up to 2.7 eggs in *P. caeruleus.* These differences apply over a period of years, and are statistically significant. The localities concerned can all be included within a circle of 40 miles radius. They differ a little in climate, as Haarlem and Breda are in the west, the other three localities in the east of Holland, where the climate is more continental in type. They also provide rather different habitats, as Haarlem and Liesboch are broadleaved woods, Driebergen and most of Hoenderlo are coniferous, and the Organisatic voor Natuurwetenschappelijk Onderzoek estate at Wageningen is partly coniferous and partly mixed conifers and broadleaved trees. The tit population is at a rather different density in the different woods, in *P. major* being highest at Haarlem, rather lower at Wageningen, and much lower at Hoenderlo and Driebergen. (In Liesboch it is not certainly known.) Kluijver (*in press*) has shown an inverse correlation between average clutch-size and population density in *P. major,* but the reason for this is not well understood. If the argument of the present paper is correct, these local differences in clutch-size depend ultimately on

Table 9. *Local differences in clutch-size in Holland. For all clutches laid April 1–May 9, 1922–36 (incl.)*

Locality	No.	Mean + S.E.		No.	Mean + S.E.	
	Parus major			*Parus caeruleus*		
Wageningen	1,042	9.01	0.05	369	10.39	0.11
Hoenderlo	443	10.26	0.08	392	10.26	0.11
Liesboch, Breda	537	10.50	0.07	314	12.33	0.12
Driebergen	203	9.02	0.12	42	9.60	0.34
Haarlem	135	7.45	0.14	59	10.09	0.28
	Parus ater			*Parus cristatus*		
Wageningen	140	8.52	0.13	49	7.12	0.17
Hoenderlo	398	8.59	0.08	249	6.88	0.07
Driebergen	180	8.63	0.12	112	7.11	0.11
Ginkel	279	8.47	0.10	104	6.73	0.11

Notes: (i) Local differences in average highly significant (1%) for *P. major* and for *P. caeruleus,* but no significant differences for either *P. ater* or *P. cristatus;* (ii) correlation coefficient between *P. major* and *P. caeruleus* + 0.594, which is not nearly high enough to be significant.

TABLE 10. *Local differences in brood-size in Switzerland for all birds banded before June 16*

Locality and years	*Parus major*		*Parus caeruleus*	
	No.	Mean	No.	Mean
Bennwil 1933–4	72	8.6	9	9.4
Allschwil 1932–3	62	8.1	29	9.3
Rümlingen 1933–4	70	8.0	14	9.1
Lyss 1931–5	228	7.6	32	7.8
Lenzburg 1935–7	102	7.6	15	7.5
Solothurn 1932–6	130	6.9	28	7.3

Correlation between *P. major* and *P. caeruleus* + .897, which is significant at 5%.

on local differences in the availability of food for the nestling tits, and until the food supply has been measured and compared in the different localities, it is not profitable to speculate further as to the meaning of the clutch-size differences.

The second section of table 9 shows that, in four selected woods (all coniferous), no significant local variations in clutch-size were found in either *P. ater* or *P. cristatus*. This does not, of course, mean that local differences are absent in these two species, but merely that they did not occur in the woods in question.

In *P. major* and *P. caeruleus*, local differences in family-size were also found in Switzerland, as set out in table 10. The six localities concerned are fairly close to each other in the low-lying parts of northern Switzerland, Lyss, Lenzburg and Solothurn being in northern Switzerland proper, Rümlingen and Bennwil in N. E. Jura, and Allschwil near Basel. Allschwil is at 287 m. above sea-level, the rest lie between 400 and 520 m. The titmice were breeding in semi-cultivated woods and orchards. Unfortunately, the Swiss data refer not to clutch-size, but to brood-size at banding, when the young are about 10 days old. Hence while they probably reflect genuine differences in clutch-size, any local differences in nestling survival will also influence the figures. Though based on rather small totals, these results have been included because the local variations found in *P. major* show a statistically significant positive correlation with those found in *P. caeruleus*. Observations in a broadleaved wood near Oxford, show that (here at least) the main food of the nestlings is the same in *P. major* and *P. caeruleus*. Hence if the local differences in the average clutch-size of titmice have adaptive significance, one would expect them to be correlated in these two species.

REGIONAL DIFFERENCES

Parus spp. also show regional differences in average clutch-size, though in view of the purely local differences that also exist, the figures must be treated with care. In passerine birds in general, there is an increase in average clutch-size from the tropics towards the poles, probably correlated with the longer feeding hours available in summer at higher latitudes (Lack, 1947). This tendency is found in titmice. Thus while the average clutch-size of *P. major major* in Holland and Central Europe generally is around 9 eggs, the various related forms breeding in the Indian region lay clutches of about half this size. Further these Indian forms themselves show a tendency for an increasing average clutch-size from south to north. Thus in southern India and Ceylon *P. m. mahrattarum* usually lays 3–5 eggs, 6 being exceptional; further north, in southern and eastern Burma, *P. m. commixtus* usually lays 4–6 eggs, 7 eggs being exceptional, though 7 eggs is regular in this form in China; further north again, in northern India, Assam and western Burma *P. m. cinereus* usually lays 5–6 eggs, sometimes 4, and 9 is on record; the most northerly form, *P. m. caschmirensis* of Kashmir, usually lays 4–6 eggs, "but the latter number is often exceeded" (Baker, 1932, evidently based on extensive data for all forms).

Data summarized in Appendix 1 also suggest that the average clutch-size of *P. major* in the Mediterranean region is slightly lower than in Holland.

Similarly in *P. caeruleus*, the usual clutch in Holland is 10–12 eggs, but

farther south, in the Mediterranean region, it is only 4–8 eggs, and still farther south, in the Canary Islands, it is normally only 3–5 eggs, rarely 6, (data in Appendix 1).

Various of the passerine species which show an increase in average clutch-size between the Mediterranean region and Central Europe show a further increase between the latter and Scandinavia (Lack, 1947). But this does not hold in most *Parus* spp. The data set out in table 11 show that in only one out of five *Parus* spp. is the average clutch-size higher in Sweden than in Holland, and that in the other four species the difference is in the opposite direction. Presumably, some factor offsets the advantage of the longer feeding hours available in summer in Sweden as compared with Holland. The same presumably applies to Scotland as compared with England, where the normal geographical trend is similarly reversed. As already mentioned, the average clutch-size of titmice is markedly influenced by local factors, and in northern Europe their effect is evidently stronger than any general geographical trend. This is further suggested by the fact that in northeastern Prussia, which is intermediate in latitude between Holland and Sweden, the average clutch-size of *P. major* seems higher than in either Holland or Sweden (data in Appendix 1, but they are not too certain, being mainly drawn from one locality over two years).

TABLE 11. *Regional differences in clutch-size (first broods)*

	Number	Mean	Standard deviation
Parus major			
Sweden	174	9.83	1.43
Holland	8,809	9.32	1.88
Scotland	61	7.36	1.67
S. England	339	10.27	1.81
Parus caeruleus			
Sweden	80	9.88	
Holland	3,455	10.65	2.16
Scotland	50	9.54	
S. England	312	11.64	
Parus ater			
Sweden	119	8.19	1.38
Holland	2,759	8.69	1.65
Scotland	50	9.06	0.96
S. England	65	9.95	1.31
Parus cristatus			
Sweden	182	4.92	.89
Holland	1,143	7.07	1.27
Parus atricapillus			
Sweden	53	7.68	1.33
Holland	346	8.24	1.71

Note: The data from England and Scotland are mainly for the years 1948 and 1949, with some for 1947. They are therefore less reliable than those for Sweden and Holland which are based on a series of years.

One factor that probably favors the titmice breeding in England as compared with Sweden is the density of the caterpillars on which they feed their young. In English oak woods the density of the defoliating caterpillars is so high that in early summer the patter of their dung on the leaves is a familiar woodland sound. It is clear from the reports of Scandinavian zoologists that such a high degree of infestation is unknown, or at least very abnormal, in their northern woods. Hence, while comparative measurements have not been made, it seems probable that the food available for nestling titmice is much less abundant in Sweden than in England.

In those passerine birds which show a south to north increase in average clutch-size, there is also a tendency for the average clutch-size to be lower in the British Isles than in Continental Europe at the same latitude (Lack, 1947). Table 11 might suggest that this trend also is reversed in titmice, but the English data were collected only over the three years 1947–49, when clutch-size was perhaps unusually high. Hence further data are desirable to settle this point. A few data on clutch-size in other parts of Europe have been added in Appendix 1.

CONCLUSION

At first sight, as least, the data in this paper do not appear to fit the hypothesis advanced in previous papers. Thus in *Parus major* a nestling from a brood of larger size has only a slightly lower chance of survival than a nestling from a

brood of smaller size. This means that those parents laying clutches of smaller size must on the average produce a smaller number of young. Yet, despite their apparent disadvantage, clutches of smaller size continue to be laid in large numbers.

If the hypothesis is correct that clutch-size has adaptive significance, the most plausible explanation of the above facts is that the smaller clutches are laid when conditions are less favorable for raising young. Field study shows that the average clutch-size of titmice is subject to unusually marked variations, both seasonal and local. For the most part it is not known whether these variations have adaptive significance, but the seasonal decline in average clutch-size in England corresponds with a seasonal decline in the caterpillar density, and the possibility that the other variations are also correlated with the food supply for the young remains open. It may be added that, while the species of *Parus* evidently possess an unusually modifiable reproducive rate, the differences between titmice and the species studied in previous papers are probably of degree not kind.

The variations in average clutch-size present not only an evolutionary, but also a physiological, problem. How, for instance, is the bird's egg-laying mechanism made aware that the season is changing? Again, it is not yet known whether the local differences in average clutch-size are due solely to a psycho-physiological response of the bird to environmental differences, or whether, in addition, natural selection has resulted in hereditary differences between local populations. The reproductive rate of titmice therefore provides a stimulating field for future research, both on the operation of natural selection in the wild, and on the influence of environmental factors on reproductive physiology.

Summary

1. The hypothesis that the normal clutch-size corresponds to the maximum number of young which can be successfully raised to maturity is tested out in European species of *Parus*.

2. Hatching success is similar for clutches of all sizes, (table 1), nestling survival is sometimes similar (table 2) but, over a long period, is slightly lower from broods of larger size (table 3). Survival after leaving the nest may be slightly lower from broods of larger size, but the difference is not significant (table 4).

3. Hence broods of larger size are not proportionately penalized. The occurrence, despite this, of many clutches of smaller size suggests that the latter tend to be laid when conditions are less favorable for raising young.

4. The average clutch-size of titmice declines markedly as the laying season advances (tables 6, 7), which can be correlated with a corresponding decline in the caterpillar population. There are marked local (tables 9, 10) and regional (table 11) differences in average clutch-size, and smaller annual differences (table 8), the significance of which is not established. Although the clutch-size of each individual varies, it is less variable than that of the population as a whole (table 5).

5. These marked variations in reproductive rates in time and space present both evolutionary and physiological problems of considerable interest for future research.

Literature Cited

Baker, E. C. S. 1932. The nidification of birds of the Indian Empire, vol. I.

Gibb, J. A. 1950. The breeding biology of the Great Tit and Blue Tit. Ibis, **92**: 507–539.

Lack, D., 1947. The significance of clutch-size. Ibis, **89**: 302–352.

Lack, D. 1948. Natural selection and family size in the Starling. Evolution, **2**: 95–110.

Lack, D. 1949. Family size in certain thrushes (Turdidae). Evolution, **3**: 57–66.

Lack, D. and Arn, H. 1947. Die Bedeutung der Gelegegrösse beim Alpensegler. Ornith. Beob., **44**: 188–210.

Appendix

Further data on regional variations

Parus major (a) *Clutch-size*

N. E. Prussia: Average of 81 first-brood clutches 10.91 standard deviation 1.64, but based on only a few seasons (calculated from the data given by Tischler, F. 1941, Die Vögel Ostpreussens.).

Ireland: Usually 7–8, often 6, rarely 9, no case of 10 in several hundred (Stoney, C. V. 1928. Some field-notes on the Irish titmice. Bull. Brit. Ool. Assoc., **2**: 64–66).

Hungary: Average of 45 clutches laid before end May 9.5 (calculated from the data from one locality over a period of years given by Warga, K. 1939. Dauer-Ehen bei *Parus m. major* etc. Jour. Orn., 87: 54–60).

Mediterranean: Usually 7–9. Corsica 1/7, 1/8, 3/9; Mallorca 1/7; Spain 1st broods 4/7, 1/9, 2nd broods 1/5, 1/6, 2/8 (unpublished field data of several ornithologists deposited at Edward Grey Institute; also Jourdain, F. C. R. 1937. The birds of Southern Spain. Ibis, 1937: 117).

(b) *Brood-size*

Note: The three following figures are within the limits for Dutch and British data.

Switzerland: Average 2,049 1st broods 7.22, and of 349 2nd broods 5.78 (Sempach data used in Table 5 D).

Hungary: Average of 1,185 broods (all dates) 8.68. Marked local differences recorded, between 4–5 in one place and 9–10 in another, and a marked decline from first broods (c. 9) to second broods (c. 6). (Csörgev, T. 1909–16. Series of reports on 'Der praktische Vogelschutz' in *Aquila* 1909–16.) A few other data from *Aquila* have been included for the general average above.

Germany: Average of 16 first broods in 1936 9.1, and of 22 first broods in 1937 8.7, average clutch-size of 10 second broods in 1938 6.6. (Berndt, R. and Frieling, F. 1939. Siedlungs- und brutbiologische Studien an Höhlenbrütern in einem nordwestsächsischen Park. Jour. Orn., **87**: 593–638.).

Parus caeruleus (a) *Clutch-size*

N. E. Prussia: Commonest size of 1st clutches 10 (average not reliable) (Tischler, *loc. cit.*).

Ireland: Usually 7–8, often 6, rarely 9, one case of 10 in several hundred, (Stoney, *loc. cit.*).

Mediterranean: 4–8 (sources as for *P. major*). (In Corsica 1/4, 1/6, 1/7, 1/8; Jourdain cites Congreve, not more than 6 in South Spain).

Canary Is.: On Tenerife usually 4, often 3 or 5, rarely 6; on eastern Canary Is. clutches of 4, 4, 5, on Palma broods of 3 and 4 (Bannerman unpubl. MS. Birds of the Canary Islands).

(b) *Brood-size*

Hungary: Average of 94 broods (all dates) 8.48 (Csörgev, 1909–16, *loc. cit.*).

Parus ater

Ireland: Much as in England, clutches of 9, 10 and 11 quite usual (Stoney, *loc. cit.*).

Parus cristatus

South Spain: Usual clutch 5, some 6 (Jourdain, *loc. cit.*).

POLYMORPHISM IN THE CHAT GENUS *OENANTHE* (AVES)

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INTRODUCTION

There is no genus of birds that could compare in polymorphic variability of coloration with certain species of lady-bugs (Coccinellidae), the grouse locusts, certain mimetic butterflies (*Papilio, Pseudacraea*), the bumble bees and other insects. Species of birds are normally either very uniform or else they show individual variability of a normal distribution. Discontinuous polymorphism in birds, if it occurs at all, usually takes the form of melanism or of albinism, that is, the occurrence of blackish or whitish individuals at regular frequencies. Even this is, on the whole, rare. A white plumage type occurs, for example, in the large genus *Accipiter* (hawks, 45 species) only in the one species *A. novaehollandiae,* and a black plumage type in the four species *A. ovampensis, erythronemius, melanochlamys,* and *albogularis.* Only in a few genera is polymorphism more widespread.

Most polymorphic variants in birds were originally described as full species, as was consistent with the typological species concept prevalent until fairly recently. Polymorphism thus became of concern to the cataloging bird taxonomist, who wanted to eliminate such genetic variants from the ranks of good species. Among birds nearly 100 so-called species have been unmasked during the last thirty to forty years as genetic variants of polymorphic species. However it was realized at an early date that these variants had a biological interest that transcended the realm of formal taxonomy. Impressed by the claims of the early mutationists, among whom de Vries and others postulated that single mutations create new species, Stresemann (1923 ff.) published a series of twenty-five "mutation studies" in which he demonstrated the widespread occurrence of polymorphism among birds. In the course of these studies, it became evident that these "mutations," as such polymorphic variants were called, had little to do with speciation. Mayr (1942) considered them as of no selective significance and implied that they were interesting but biologically insignificant accidents of nature.

The solution of the problem of the real significance of polymorphism came from a different direction. During a study of gene frequencies, R. A. Fisher (1930) showed that an allele would eventually replace other alleles in a population, provided it had some selective advantage, no matter how small. On this basis one would have to expect either that the selective values of various polymorph alleles are identical, which is exceedingly unlikely since the polymorph variants are the visible, phenotypic expressions of different gene-physiological processes, or that we are observing a temporary stage of "transient polymorphism," during which one allele is gradually replaced by the other. The facts militated against the correctness of this second assumption. Fisher showed that there is a third alternative. The balance of the gene frequencies need not be a static one: If the selective value of the heterozygote is superior to that of either homozygote, a condition of "balanced polymorphism" will result. Much genetic work during the past twenty years has confirmed this prediction. The study of polymorphism in birds has, thus, gained a new significance.

Among polymorphic genera of birds, the genus *Oenanthe* (with about 18 species) is outstanding for the diversity of polymorphism.

List of species of Oenanthe

pileata Gmelin 1789
tractrac Wilkes 1817
bottae Bonaparte 1854
**xanthoprymna* Hem. and Ehr. 1828
moesta Lichtenstein 1823
isabellina Cretzschmar 1826
oenanthe Linnaeus 1758
**pleschanka* Lepechin 1771
**hispanica* Linnaeus 1758
deserti Temminck 1825
finschi Heuglin 1869
**picata* Blyth 1847
**lugens* Lichtenstein 1823
monacha Temminck 1825
alboniger Hume 1873
leucopyga C. L. Brehm 1855
leucura Gmelin 1789
**monticola* Vieillot 1818

Polymorph species with asterisk.

The genus *Oenanthe* belongs to a group of thrushes which is usually referred to as chats. It is only poorly delimited against other genera of chats (e.g., *Cercomela, Emarginata, Karrucincla, Pinarochroa*), having as chief character the white or rusty color of rump and base of tail. There is usually a well-defined black terminal or subterminal bar across all tail-feathers. Complete agreement on the exact number of species does not exist because certain allopatric forms are considered conspecific by some authors and different species by others, as for example: (1) *lugens-lugubris,* (2) *tractrac-albicans,* (3) *oenanthe-phillipsi,* (4) *lugens-picata,* (5) *bottae-isabellina,* and (6) *moesta-xanthoprymna.* We consider the first three pairs of forms as conspecific but not the others. The species *pileata* is so different from the normal color pattern of the genus that it will not be included among the eighteen species discussed by us in more detail. All these species are either largely black and white or sand-colored (or gray) to a greater or lesser extent. The sandy coloration seems to be secondary rather than the primitive dress of the genus. The species *isabellina* and *tractrac* are the extremes of this trend. Reduction of the color pattern is less far advanced in *oenanthe* and *deserti.* At the other extreme are several species that are entirely black and white (in various combinations) without any sandy coloration. This is true particularly for *monacha, alboniger, leucura, leucopyga, lugens,* and *picata.*

Polymorph Characters

Six species of *Oenanthe* (*monticola, lugens, picata, hispanica, pleschanka,* and *xanthoprymna*) have polymorphic populations. The character most frequently involved is the coloration of crown, throat, or abdomen.

Crown

The crown is colored like the rest of the upperparts in nearly all the species of *Oenanthe.* If the back is black, as in *alboniger* and *leucura,* the crown is also black; if the back is gray or sand-colored, then the crown likewise is gray or sand-colored.

Exceptions are a number of black-backed species in which the crown is white (or pale gray). These species are *monacha, lugens, pleschanka,* and *moesta.* In addition there are three species which, according to the literature, are polymorphic in this character: *monticola, leucopyga,* and *picata.* However, a study of our series of *leucopyga* shows that true polymorphism does not exist in this species. All black-headed birds are in the first-year plumage; all white-headed birds are adult. This leaves true polymorphism of the color of the crown for two species: *monticola* and *picata.* In both species the light color of the crown is already visible in the first-year plumage. In both species there is geographical variation in the frequency of this character.

Table 1. *Frequency of black and white crown in various parts of the range of* Oenanthe monticola

	Black head	Light head
South Africa (A.M.N.H.)*	4	1
Griqualand (Davies)	common	scarce
Brandberg (West Damaraland) (Niethammer *in litt.*)	40	0
Southwest Africa (A.M.N.H.)	0	1
Benguella (Angola) (A.M.N.H.)	4	8

* A.M.N.H. = Collections of the American Museum of Natural History.

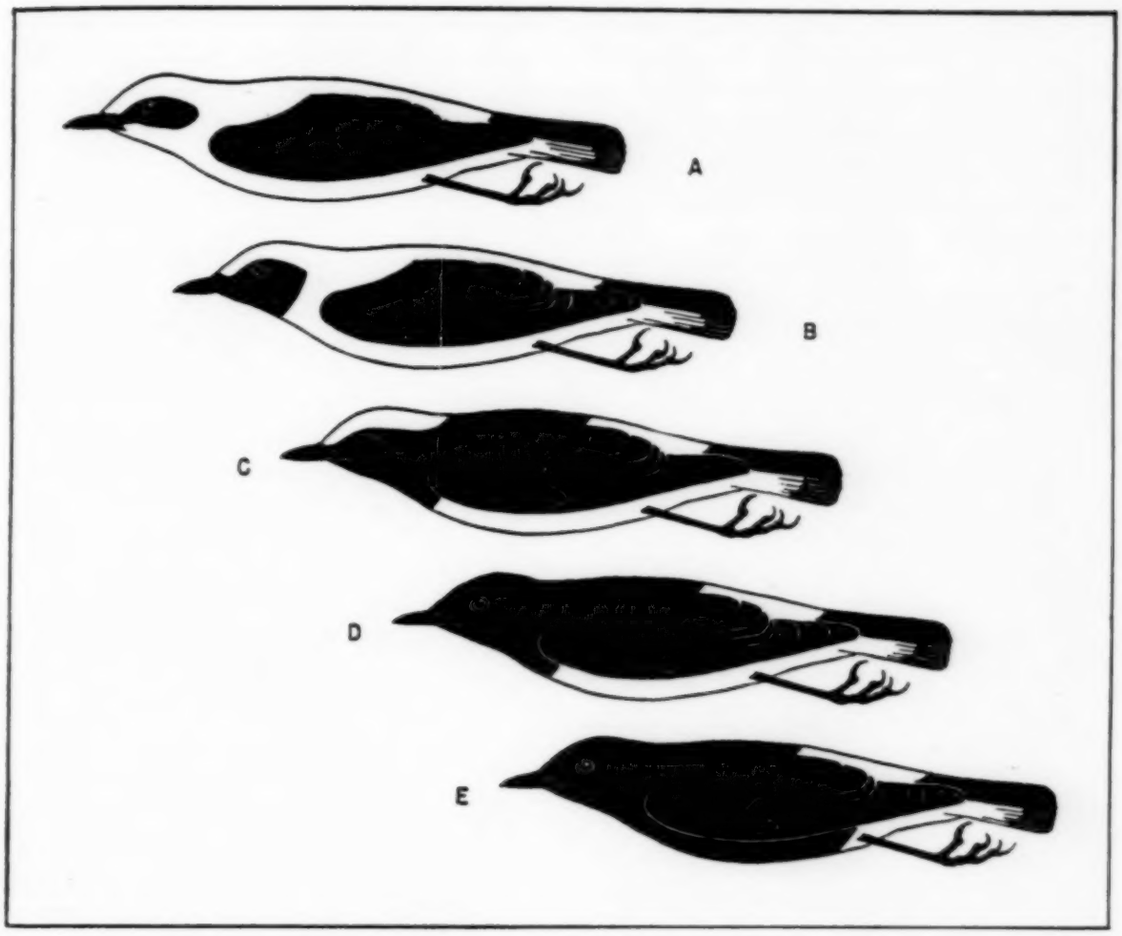

FIG. 1. Polymorphism in *Oenanthe*. A, B. *Oe. hispanica*. C, D, E. *Oe. picata*, C = *capistrata* type, D = *picata* type, E = *opistholeuca* type.

The available material of *monticola* is not sufficient to give reliable quantitative data on frequency of the two types and their geographical variation. It is listed in table 1.

The light head is gray in South Africa and white in adults from Benguella (gray in immatures).

There is also a factor in this species that produces a gray plumage. Among 23 examined specimens only 3 from South Africa and 2 from southwest Africa are in this plumage. It is common in Griqualand (Davies, 1910), but not represented among 40 males from West Damaraland or 12 males from Angola.

In *picata* the crown is usually black, but a white-headed form occurs which was originally described as a separate species (*capistrata*). That this is a case of polymorphism has been demonstrated by several authors, particularly by Stresemann (1925) and Vaurie (1949). The *capistrata* type is absent in Iran and in Afghanistan south of the Hindukush; it is rare in Kashmir and adjacent parts of northwestern India. In northern Afghanistan and particularly in northern Tadzhikistan (see fig. 2) there seem to be entire populations consisting entirely of *"capistrata"* individuals (see below).

Belly

The abdomen is light colored in nearly all species of *Oenanthe*. It is black in two species (*leucura, leucopyga*) and polymorphic in three others (*lugens, picata,* and *monticola*).

In *lugens* the abdomen of adult males is normally black, but the males from cer-

tain parts of the Abyssinian highlands are dimorphic; they may be either black-bellied or white-bellied (Vaurie, 1950).

In *picata* the form *"opistholeuca"* is black-bellied. It is absent—or very rare—in Iran and northwest India, but is frequent in northeastern Afghanistan. Among 31 specimens from northeastern Afghanistan 16 were *opistholeuca* (Vaurie, 1949). In eastern Tadzhikistan (slopes of western Pamir) the frequency may be even higher. In certain localities, par-

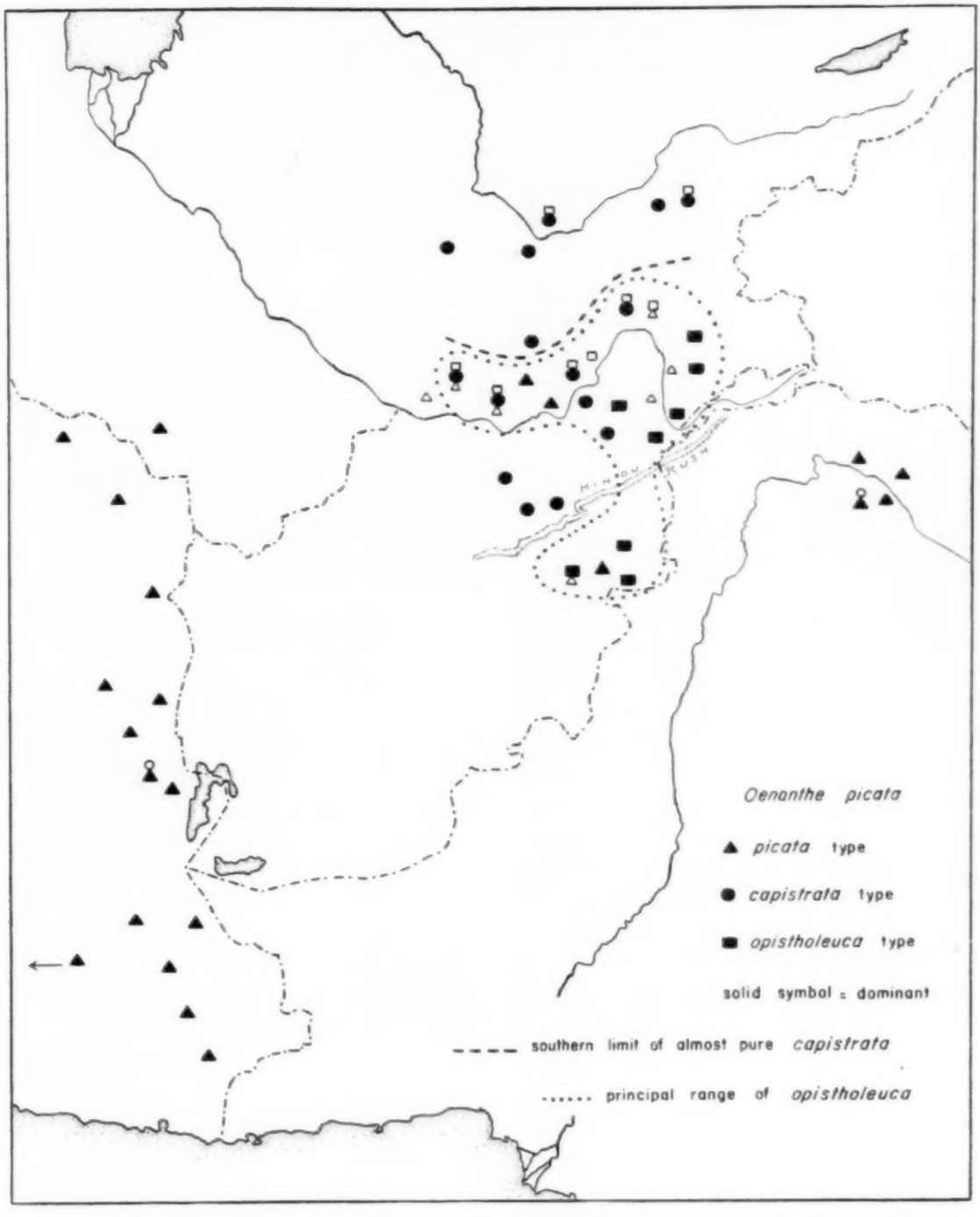

FIG. 2. Distribution of the three forms of *Oenanthe picata*. Empty symbols indicate occurrence at low frequency. The dotted line encircles the chief range of the black-bellied form (*opistholeuca*). East (Kashmir) and west (Iran) the form *picata* reaches a frequency of 90–100 per cent.

ticularly at the higher altitudes (1,400 m.–2,500 m.), it is the only form found (Ivanov, 1940).

In *monticola* of South Africa, white-bellied birds appear to be most common in the north and get rarer toward the Cape. Only 1 of 12 Benguella birds is black-bellied. In Western Damaraland white-bellied birds are only slightly more frequent than black-bellied individuals (Niethammer, *in litt.*). Among 5 birds from southern South Africa 3 are white-bellied. Davies (1910) stated that in Griqualand birds with black bellies are much more common than with white bellies. There is thus indication of a definite cline, but neither form seems to reach anywhere a frequency of 100 per cent.

Throat

The throat is black in the adult males of nearly all species of *Oenanthe*, even in many species with pale upperparts. The only exceptions are the two somewhat aberrant African species *tractrac* and *bottae* and the sandy species *isabellina*, and *oenanthe* and *xanthoprymna* in the greater part of their ranges. In the latter two species there are some geographical races with a black throat, the nominate race of *xanthoprymna* (Iraq) and the two subspecies *seebohmi* (north Africa) and *philippsi* (Ethiopia) of *oenanthe*. In the case of *xanthoprymna* both black- and white-throated birds occur in a zone in western Iran where the black-throated *xanthoprymna* and the pale *chrysopygia* meet (Vaurie, 1949). Although this is, strictly speaking, more a case of hybridism than of polymorphism, the black-throated and light-throated types in the "hybrid" population are well defined and without intermediates. Throat color is apparently determined by a single gene.

The situation is even more clearcut in two other species, *hispanica* and *pleschanka*, in which "black throat" is a polymorphic character. These two species are very similar and almost entirely allopatric. Some authors have even gone as far as considering them conspecific. However, as Grote (1939) and Vaurie (1949) have shown, the breeding ranges overlap in eastern Asia Minor, Transcaucasia, and western Iran. In the collections of the American Museum males of *hispanica* show the following ratio of black-throated individuals (Vaurie, 1949).

TABLE 2. *Frequency of black-throated males in* Oenanthe hispanica

	Number of males	Per cent black-throated
Southeastern Europe	11	54.5
Palestine	24	62.5
Iran	39	64.0

For comparison the following figures from the literature are added:

Macedonia (Stresemann, 1920)	23	52.0
Egypt (migrants) (Meinertzhagen, 1930)	68	62.0

The ratio for populations from the western Mediterranean is as follows:

	Number of males	Number	Per cent
		Black-throated	
Southern France and Spain	7	(5)	(71)
Morocco	35	18	51.5
Algiers	25	11	44
Total	67	34	50.7

	Number black-throated	Number white-throated	Per cent black-throated
Total eastern Mediterranean	100	65	60.6
Total western Mediterranean	34	33	50.7

$\chi^2 = 1.9 \quad P = .18$

These figures show that all larger samples of this species have about 50 per cent or more black-throated individuals. Nowhere does one of the two forms gain complete ascendancy over the other one.

However, black-throated individuals occur in the eastern Mediterranean with a higher frequency, although the samples are too small to indicate significance in an χ^2 test. This trend is continued within the eastern populations since birds from the Balkans have 18 black-throated individuals among 34 (53 per cent), while birds from Palestine, Iran, and Asiatic migrants in Egypt have 82 black birds among 131 (62.5 per cent), $\chi^2 = 1.85$.

This indication of an increase of black-throated birds toward the east is interesting since in *pleschanka,* the species which (with some overlap) represents *hispanica* in the east, nearly all individuals are black-throated (97.8 per cent). Of 228 *pleschanka* males examined only 5 are the light-throated *"vittata."*

The Genetics of Polymorphism

Nothing is known as to the genetics of these color phases, except that they appear to be complete dominants. They are, however, in each case so clearly alternative characters that there can be no doubt that they are caused by single genes or chromosome sections. They are never connected by a series of intermediates. There is some evidence for modifiers, for example, the white crown of *"capistrata"* (in *Oenanthe picata*) is usually pure white but darkened (*"evranovi"*) in a few individuals in some areas.

Little is known of linkage in the multiple polymorph species. In *picata,* black belly (*"opistoleuca"*) seems to occur only in black-headed individuals. In *monticola,* on the other hand, black-bellied and white-bellied individuals occur in both types of head coloration.

Discussion

The special importance of geographically variable polymorphism is that it sheds new light on the potential significance of seemingly neutral taxonomic characters. Since birds are unsuitable for purposes of population genetics, the interpretation must be based on analogy with similar situations in insects that are accessible to genetical analysis.

The differences between species of birds are very often of a gradual quantitative kind as between most species of warblers of the genera *Phylloscopus* or *Sylvia.* Conspicuous differences in color patterns exist in other cases, such as those between the nuptial plumages of adult males of various species of American wood-warblers (*Dendroica*). These pattern differences are commonly interpreted as being without biological significance except as "recognition signals" in courtship. A study of polymorphism indicates that pattern differences may have a selective significance that goes beyond its function in courtship.

This conclusion is strengthened by a study of geographical variation, which in continental bird species is usually of a clinal, quantitative type. Populations are darker or paler, more rufous or more olive, of larger or smaller size. A discontinuous type of variation—e.g., throat white or throat black—occurs in continental birds normally only in species with well isolated (insular!) subspecies. The genus *Oenanthe* is exceptional in the frequency of discontinuous variation. This is true on three taxonomic levels, in regard to differences between individuals (polymorphism), between subspecies (geographical variation), and between species (species characters).

Intrapopulation Polymorphism

Polymorphism cannot be understood until one realizes that the polymorphic character and the gene that causes it are not one and the same thing. The variable character itself, e.g., "black throat," vs. "white throat," will only rarely have selective value. However, the phenotypical character is merely the visual expression of a gene that controls various invisible physiological processes, which may have positive or negative selective values. Two alleles can coexist in the same population for any length of time only under two conditions. Either they are exactly alike

in their selective values—the occurrence of such selective neutrality is exceedingly unlikely, as pointed out by R. A. Fisher, E. B. Ford, and others—or else the heterozygote is superior to either homozygote. This selective advantage of the heterozygotes will tend to preserve the inferior allele and result in a case of balanced polymorphism. Heterozygous adults will be more frequent in such a population than expected according to the Hardy-Weinberg equilibrium.

R. A. Fisher (1939) demonstrated this for the first time for a wild population (the grasshopper *Paratettix*) and many additional proofs for single genes as well as for gene arrangements have been furnished since (Freire-Maia, 1949, Dobzhansky and Levene, 1948, Wallace, 1948, de Cunha, 1949, etc.). Two results of this genetic work are outstanding: (1) The differences in the selection coefficients of gene arrangements and even of alleles are of a much higher order of magnitude than had been expected, (2) An apparently insignificant morphological character (such as the color of the abdomen in *Drosophila polymorpha*) may be caused by a genetic condition that affects simultaneously a considerable number of viability factors, such as longevity, sexual activity, fecundity, resistance to desiccation or to high or low temperatures, etc. As emphasized above, polymorphism has gained new significance in the light of these studies.

Geographically Variable Polymorphism

The external, as well as the genic, environments are different for every population of a species. It is to be expected that the relative values of the selective advantage of the two homozygotes and the heterozygote of a polymorphic gene change from place to place. The researches of taxonomists have fully confirmed such an expected geographical variation of polymorph ratios (Mayr, 1942; Ford, 1945). In nearly every case of polymorphism in the genus *Oenanthe* there is geographic variation in the frequency of the two alleles. As described above it affects the characters of light head, black belly, and gray plumage in *monticola*, black belly in *lugens*, white crown and black belly in *picata*, and light throat in *hispanica*. The frequency of the character of light throat in *pleschanka* is so small that it is difficult to prove geographical variability.

Both alleles are found throughout the entire range of the species apparently in only two of all cases of polymorphism in *Oenanthe: monticola* (white crown and black belly), and *hispanica* (white throat). In all other cases, one of the two alleles becomes the exclusive gene in large parts of the species range.

These cases, such as the apparent absence of the gray plumage of *monticola* from Damaraland and Angola, lead to a new appreciation of discontinuous subspecies characters (see also Mayr, 1942, fig. 9). One may assume by analogy with the above quoted experimental results that a fixation of one of several characters (in a definite geographical region) is due to a shift in selective values. Fixation of one of the alleles will develop from balanced polymorphism wherever one of the homozygotes becomes superior to the heterozygote. In *O. xanthoprymna*, for instance, the two eastern subspecies (*chrysopygia* and *kingi*) are light-throated but intergrade with the western black-throated subspecies *xanthoprymna* in a narrow belt occupied by a polymorphic population.

In *picata* there are several portions of the range in which only one of the three forms occurs. In Iran, for instance, only *picata* is found at most localities (Vaurie, 1949), in northern Tadzhikistan only *capistrata*, and in the western Pamir at higher altitudes only *opistholeuca* (Ivanov, 1940) (fig. 2). The "pure" *picata*, *capistrata*, and *opistholeuca* districts are connected by mixed belts. In *O. oenanthe* all northern populations are white-throated, but the subspecies *seebohmi* (north Africa) and *philippsi* (Somaliland) are black-throated. In these cases

characters have become subspecies characters, which in other species are polymorph characters. It would not be unreasonable to assume that homologous genes might be involved.

Species Characters

What makes the genus *Oenanthe* particularly interesting is the fact that the same characters that are polymorph characters in one species occur as subspecies characters in other species, and finally may also occur as species characters. The color of the belly, which is polymorph in *lugens, monticola,* and *picata,* is light in most species of the genus, but black in *leucura* and *leucopyga.* The throat is black in the majority of the species, polymorph in *hispanica, pleschanka,* and *xanthoprymna,* geographically variable in *xanthoprymna* and *oenanthe,* and light in *tractrac, bottae, isabellina,* and a few of the aberrant species. The color of the crown in black-backed species may be black (*alboniger, leucura*), polymorph (*monticola, picata*), or white (*monacha, lugens, pleschanka, moesta*).

The frequently made assertion that the color characters of species of birds are accidents of variation and without selective significance appears dubious in view of the fact that so many of these characters are either involved in balanced polymorphism or subject to geographical variation or both.

Selective Value of Different Polymorph Characters in Different Habitats

There is an interesting correlation between habitat differences and shifts in polymorph frequencies. In *O. xanthoprymna,* for example, the black-throated *xanthoprymna* is found in Iraq in the hot foothills, while the white-throated *chrysopygia* is found above the escarpment in the Zagross Mountains and on the Iranian Plateau. A polymorph belt occurs where the two habitats meet.

In *picata* there is a sharp change of pattern frequencies along the line of the Hindukush range. South of it only *picata* and *opistholeuca* are found, north of it *picata* becomes rare and *capistrata* becomes the dominant form. In north Tadzhikistan almost nothing but *capistrata* is found. In western Pamir both *picata* and *opistholeuca* occur, but at higher altitudes *opistholeuca* exclusively. Clinal changes of the pattern frequencies occur also in *monticola.*

The available data do not permit an accurate correlation between given genes and precisely determined ecological conditions. That such a correlation exists is, however, no longer doubtful. It is also substantiated in other genera. The dipper (*Cinclus cinclus*) occurs in two color types at the border of Tibet and western China. In the forest belt of the Yangtze Valley only white-breasted birds occur. Toward the edge of the Tibetan plateau there is a steady increase of the brown-breasted form. Schäfer (1938), on his way from China to Tibet, encountered the first brown-breasted bird at 4,100 m. on a tributary of the Dichu (upper Yangtze). "As we entered the tree- and shrub-less high steppes, the dark-breasted *Cinclus* became quite common and outnumbered by far the white-breasted individuals which occurred only sparingly. A few white-breasted individuals are found throughout the high steppes while a few brown-breasted birds occur anywhere in the wooded zone along the tributaries of the Yangtze. On the upper Yangtze itself I have never seen a brown-breasted bird, there only the white-breasted form occurs." A similar distribution of the two color forms of *Cinclus* in the various ecological zones of the China-Tibet border area has been described by other travelers. Since white-breastedness is the "normal" coloration of this far-ranging species, one may postulate that the allele for brown-breastedness is associated with special survival qualities on the climatically severe high steppes of central Asia.

The Nature of Selective Differences

If the heterozygote (when phenotypically indistinguishable from the dominant

homozygote) is selectively superior, this indicates that not the visible phenotype ("taxonomic character") but the underlying gene controls the selective value. Nevertheless, there are some facts available that indicate that some of the visible properties of the color pattern in *Oenanthe* may also be of selective influence.

While most desert birds have cryptic coloration, it is remarkable that so many species of *Oenanthe* have a plumage pattern composed of the contrasting colors black and white. Cott (1947) has shown that their flesh is distasteful and that the conspicuous coloration apparently serves as a warning coloration. The black-and-white species are, on the whole, associated with cliffs and rocky terrain in the hot deserts. The two species that extend farthest into the north temperate zone (*oenanthe* and *isabellina*) have the black markings reduced or absent. It is not obvious what the climatic relation could be.

There is some evidence for a connection between endocrines and color pattern. *O. leucopyga* occurs in two plumages; the crown is either black like the back or pure white. This is usually interpreted as genetic polymorphism, such as found in *picata* (*"capistrata"*). However, a closer analysis of plumage changes and molts in 80 specimens has shown that all black-headed birds are in the first-year plumage and all white-headed birds are adult. Colonel Meinertzhagen has confirmed these findings for over 120 specimens in his collection and that of the British Museum. That black-headed birds may molt in this species into the white-headed plumage had already been found earlier by Lynes (1925) and Arnault (1926). It is interesting that hormones should control in this species a change of a color pattern which in other species of the genus is genetically determined, either as a species character or as polymorphism.

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Summary

(1) The chat genus *Oenanthe* with eighteen species shows much polymorphism of color pattern in six species.

(2) The variation is on the whole strictly discontinuous (e.g., black throat "present" or "absent"). It is, therefore, presumably controlled by a single gene, switch gene, or linked chromosome section.

(3) The frequency of the two alleles shows much geographical variation which either forms a regular cline or is irregular. The frequency of one of the alleles reaches 100 per cent in parts of the geographical range of five of the six species.

(4) Since recent genetic evidence indicates that the alleles involved in balanced polymorphism have different selective values, it seems probable that many subspecies and species characters that have heretofore been considered as "neutral" actually differ in their selective values.

Literature Cited

Arnault, Ch. 1926. Le traquet à tête blanche. L'Oiseau, **7**: 156–160.

Cott, H. B. 1947. The edibility of birds. Proc. Zool. Soc. London, **116**: 371–524.

Cunha, A. B. da. 1949. Genetic analysis of the polymorphism of color pattern in *Drosophila polymorpha*. Evolution, **3**: 239–251.

Davies, C. G. 1910. Notes on the plumage of the Mountain Chat (*Saxicola monticola* Bechst.). Jour. South African Ornith. Union, **6**: 33–37.

Dobzhansky, Th., and H. Levene. 1949. Genetics of natural populations. XVII. Proof of operation of natural selection in wild populations of *Drosophila pseudoobscura*. Genetics, **33**: 537–547.

Fisher, R. A. 1930. The genetical theory of natural selection. Clarendon Press, Oxford.

——. 1939. Selective forces in wild populations of *Paratettix texanus*. Ann. Eugenics, **9**: 109–122.

Ford, E. B. 1945. Polymorphism. Biol. Rev. **29**: 73–88.

FREIRE-MAIA, N. 1949. Balanced polymorphism in *Drosophila montium*. Evolution, **3**: 98.

GROTE, H. 1939. Ist *Oenanthe pleschanka* (Lepech.) eine Rasse von *Oenanthe hispanica* (L.)? Ornith. Monatsber., **47**: 54–57.

IVANOV, A. J. 1940. Oiseaux du Tadzhikistan, Moscou, pp. 252–255.

LYNES, H. 1925. Birds of north and central Darfus. Ibis, **67**: 116.

MAYR, E. 1942. Systematics and the origin of species. Columbia University Press, pp. 73–82.

MEINERTZHAGEN, R. 1930. Nicoll's Birds of Egypt, **1**: 258–281.

SCHÄFER, E. 1938. Ornithologische Ergebnisse zweier Forschungsreisen nach Tibet. Jour. Ornith., Sonderheft, pp. 205–207.

STRESEMANN, E. 1920. Avifauna Macedonica, p. 167.

——. 1923 ff. Mutationsstudien. (For full list see Jour. Ornith., **74**: 377, 1926.)

——. 1925. *Oenanthe opistholeuca* (Strickland)—eine melanistische Mutante von *Oenanthe picata* (Blyth). Ornith. Monatsber., **33**: 178–181.

VAURIE, C. 1949. Notes on the bird genus *Oenanthe* in Persia, Afghanistan, and India. Amer. Mus. Novitates, no. **1425**: 1–47.

——. 1950. Variation in *Oenanthe lugubris*. Ibis, **92**: 540–544.

WALLACE, B. 1948. Studies on "sex ratio" in *Drosophila pseudoobscura*. I. Selection and "sex ratio." Evolution, **2**: 189–217.

EVOLUTION AND PLUMAGE TRAITS IN PHEASANT HYBRIDS, *PHASIANUS* × *CHRYSOLOPHUS*

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INTRODUCTION

It has been amply demonstrated that hybridization affords an effective means for analyzing some of the problems that confront students of evolution and systematics. One such problem concerns the nature of genetic complexes back of those traits which serve to distinguish between related forms and another is the frequently encountered "reticular" distribution of traits that makes it possible to group the same array of organisms in several different ways; e.g., A, B, and C as AB–C, AC–B or A–BC. Such an apparent classificatory triad might reflect a misinterpretation, or it might be explained by convergent evolution, by enduring effects of past hybridization, or by the phylogenetic persistence of traits which early in the process of speciation became sorted more or less at random. There is always the possibility that hybridization may partially break down some of the established complexes and render their elements more accessible to analysis. While work of this sort can usually be carried out more quickly and efficiently with rapidly reproducing lower forms, it nevertheless is important to secure data whenever possible from higher animals, even though the process is more laborious and the results less numerous.

Various interspecific and intergeneric hybrdis have been studied in the pheasant family, Phasianidae, especially by Haigh Thomas ('14) and her associates, by Ghigi ('37), and by others, to the end that a considerable volume of data on first and second generation hybrids has now accumulated. Such data relate mostly to the question of sterility and the manifestation of some of the more striking somatic traits. Darwin's work on the origin of the domestic fowl has become the classic example in this field of the attempt to draw evolutionary inferences from hybrid characteristics. In the course of some earlier endocrine studies three distinct species of pheasants were encountered which can be hybridized and still show enough fertility among the male offspring to make it possible to carry the experiment on into later generations. This suggested the desirability of a detailed examination of characters which can be traced in the parent species and in successive generations of hybrids. Since such an analysis involves attention to a number of minor details, the introduction of a certain amount of descriptive material unfortunately becomes necessary.

For the purpose of the study, hybrids were obtained from *Chrysolophus pictus* × *C. amherstiae;* one of these hybrids was crossed to *Phasianus,* and the male offspring back-crossed to *Chrysolophus* for several successive generations. The original *Phasianus* stock included representatives of two types, (1) Mongolians obtained from the State Game Farm at Yountville, California, apparently typical *P. colchicus mongolicus,* and (2) mutants (*P. c.* mut. *tenebrosus*) obtained from a private breeder and found to be homozygous. The original *Chrysolophus* stock came from several commercial breeders and had every appearance of being genetically pure. All hybrids were raised from

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eggs laid and incubated at the laboratory. A preliminary report on the first intergeneric cross was made by Danforth and Sandnes ('39). The latter author has in preparation a detailed study of sterility and some other traits appearing in later generations. The present paper will be restricted to consideration of a few characteristics of the plumage with reference to evolutionary differences and speciation within this limited group. It is assumed that the three species are probably closely enough related so that the same chromosomal loci are present in all of them.

Sexual dimorphism in pheasants is so great that it has seemed best to limit this discussion to feathers of the males only. Plumage differences between the sexes are normally conditioned by both genic and hormonal factors (Danforth, '42), and the effects of neither can be studied adequately without regard to the other. In this work the effects of hormonal factors have been standardized so far as possible by using only fully mature, well-nourished males in adult plumage. *Phasianus* males acquire this plumage fairly early, but *Chrysolophus* males do not attain theirs until well into the second year. Even with the field thus restricted the number of significant morphological and pigmentary features has proved to be so great that scarcely more than an abstract of the accumulated data can be presented in the available space.

Two classes of pigment are present, yellows and reds, which are soluble in hot HCl, and melanins which are not. The latter (really dark brown in color) are presumably the same in both genera and, depending on the arrangement of the granules and the structure of the feather in which they occur, give the various tones of violet, purple, blue, green, some browns and gray. The red and yellow pigments are more numerous and, at least to some extent, chemically different in the two genera, as they are in species of *Colaptes* (Test, '42). In the developing feather they appear to be granular, but the granules either fragment completely (Bohren *et al.*, '43) or actually dissolve into the feather substance. The best available evidence indicates that the primordial pigmentoblasts are potentially capable of producing soluble or insoluble pigments depending on what particular hormonal and other factors prevail at some critical moment in the development of any individual feather. In this connection it may be noted in passing that while *pictus* and *amherstiae* can both produce the same red, its distribution in the plumage is different and when it does occur in homologous regions its intensity is not the same in the two species. The quality of some of the colors is so elusive that they cannot be satisfactorily designated by other than vernacular terms and circumlocutions.

Except as otherwise indicated, the terminology applied to feathers is essentially the conventional one, which to a considerable extent dates back to Nitzsch (1840). The tip of a feather is here considered as distal, the margin nearer the mid line of the bird as medial. Barbs are thought of as oriented with reference to the feather as a whole, barbules with reference to the barb to which they are attached. Barbules pointing toward the rachis are referred to as medial, those pointing toward the margin of the feather as lateral, which means that each feather has rows of both "medial" and "lateral" barbules on either side of its axis. Very heavy sub-terminal barbules whose modified form and pigmentary content produce iridescent effects are here designated as *iridophores*. A cluster of modified barbules with long curved teeth at the end of a barb is called a *terminal brush*. Barbs without barbules are called *free* and designated as *keeled* or *crested* in case there is a marked thickening on their inner or outer side. P is used throughout for *Phasianus*, A for *Chrysolophus amherstiae*, G (Golden) for *C. pictus* and C for *Chrysolophus* without regard to the species. Pedigrees are indicated by combining symbols; e.g., GA for *pictus* × *amherstiae*, GAG for *pictus* × *amherstiae* back-crossed to *pictus*, PCCC for *Phasi-*

anus × *Chrysolophus* back-crossed to *Chrysolophus* for two successive generations.

Description of Hybrids

Hybrids between P. c. mongolicus *and* P. c. tenebrosus

This cross will not be considered in detail. Briefly, *mongolicus* is a large brightly colored "ringneck" and *tenebrosus*, a more uniformly colored melanotic form. Nearly all feathers of both sexes are differently colored in the two forms. Hybrids are slightly less melanotic than pure mutants, show a faint trace of a neck ring and have sides, wings and tails that show some trace of the Mongolian ancestry. Superficially, however, they would ordinarily pass as *tenebrosus* (which in the wild is said to have two phases—doubtless the pure and the heterozygous). Our data, those of Bruckner ('39) and of others leave little doubt that the main *tenebrosus* character is conditioned by a single gene mutation. Since this gene was lost in our first intergeneric cross and has not been reintroduced, it does not enter into the make-up of the following hybrids.

Hybrids between the two species of Chrysolophus

Except for a few unspecialized feathers, G and A males, like mutants and Mongolians, differ throughout the entire extent of their plumage. Although they also differ in body size, skin color and many other features, hybrids between the two species are common. They are said to be completely fertile (Phillips, '21), and in our relatively small numbers we have found no indication of sterility barriers tending to isolate the two species, but neither our data nor those in the literature would justify a categorical statement that none whatever exists. (In nature the ranges of the two species are said to be separated by a gap.) The specimens considered here are from a cross between G male and A female and from the back-cross F_1 female to G male.

Inter-generic hybrids

Most of the hybrid specimens to be described are descended from a single male bird derived from the mating of an F_2 Mongolian × mutant male and an F_1 G × A female. By comparison with his sibs it is clear that this male did not receive the *tenebrosus* gene and therefore, so far as coloring is concerned, may as well have had no *tenebrosus* ancestry. The male, PC (i.e., ½P, ½C), was mated to two females, an F_1 from a cross between an A male and a G female, and an F_2 from a reciprocal cross. Unfortunately, again in the next generation only one available male was produced. This second male, which according to the older reckoning would be counted as ⅞P, ⅜A and ⅜G, was likewise mated to two females, one pure G, the other ⅞G. All subsequent matings were between hybrid males and G females until after the fifth generation, when the appearance of some fertile female young made other types of mating feasible. It may be recalled here that it is impossible to know the exact genotype of any hybrid after the first generation except as individual traits can be identified by inspection or checked genetically. On the whole, statistically the most probable distribution of genes may be assumed to be that indicated by pedigrees expressed in the familiar fractional form.

Plumage Characteristics of the Species and their Hybrids

Two of the regions will be described in some detail, the others more briefly.

Pileum

The feather-bearing area on the top of the head may be divided into three regions: the forehead, extending from the nostrils back to the level of the base of the nasal and prefrontal bones (about equivalent to a line between the two inner canthi of the eyes); the frontal region (crown), from this line back to the level of the fronto-parietal suture; the parietal region (occiput), from the preceding su-

ture back to that between the parietal and occipital bones.

G. In G all the feathers of these three regions are highly specialized and of the same general type. They have a restricted plain yellow web, followed by a greatly elongated, canary yellow, shaft and barbs, most of which are entirely free of barbules except at their proximal attachments. Throughout the free portion there is a high triangular crest which in cross section of the barb gives the effect of an isosceles triangle with a rounded base. Most of the barbs have pointed, awl-shaped tips, but a few have brush tips. It is these long free barbs which give justification for Beebe's ('26) description of the crest feathers as "hairy." In length, there is an even gradation from 12 mm or less on the forehead to as much as 80 mm in the mid-parietal region. Tips of the longest feathers reach to the lower fourth of the cape.

A. In the parietal region feathers of A are morphologically similar to those of G, except that their maximum length is some 20 mm less, almost all the barbs have swollen tips, and not only the tips, but the barbs as a whole are bright red. Terminal brushes occur, but they are fewer and less well developed than in G. When present they may contain a few melanin granules. In its total effect, the crest appears as a bright red triangle whose base extends across the parietal region from one circumocular bare area to the other, and whose apex reaches a point about halfway down the cape. If these red feathers are soaked for an hour in half normal NaOH and the resulting bright yellow fluid poured off, acidified and boiled, a clear port wine colored liquid is obtained which for convenience may be called C-red. This C-red can be obtained from other red feathers in A and G, but not from the yellow feathers nor from any feathers in P. The rest of the crown and forehead in A is covered with feathers more nearly of the ordinary sort and essentially similar to those of P, from which they differ in being somewhat less robust, in having much white in their undercolor, and in reflecting a clearer shade of green.

P. Feathers of the forehead are about 8 mm long with a dark blue-green sheen in their exposed portions. The afterplume is relatively long, and the main feather has a short web distal to a dull black fluff. Toward the end of each feather the barbules become heavier and are closely appressed to their respective barbs. The barbs themselves are not free at any point but these closely appressed barbules create the impression of free diverging rays such as actually do occur in G. Further back over the frontal and parietal regions similar feathers reach a maximum length of about 30 mm. In the latter region there are small tufts of slightly differentiated erectile "ears" just above the postero-dorsal corners of the circumocular area. The reflected color is a pale green with the appearance of a grayish wash which is created by the lightly pigmented rachides and basal parts of the iridophores.

GA. As in G, feathers of the nasal, frontal and parietal regions form a single unified crest, but the lengths of all feathers are intermediate between those of the parent forms, 18 mm on the forehead, 70 mm in the parietal region. In morphological details, feathers of the parietal region where the parents are similar show nothing new, while those on the forehead and crown are entirely different from A but similar to G except for reduction in size. In over-all color, feathers of the whole region are generally only slightly less brilliant than those from the parietal region of A. In one case they were somewhat lighter. The undercolor of the larger feathers shows some resemblance to A in both tone and markings. Apparently both A and G pigments are present in all the feathers of the crest. Many of the barbs have swollen tips.

PC. The intergeneric hybrid has feathers with a maximum length of 17 mm on the forehead (about equal to GA) and 30 mm in the parietal zone (the same as P). Their color is a new, moderately bright

rufous brown with a somewhat vinaceous sheen. Unexpectedly, it was found that a trace of C-red could be extracted from these crest feathers, suggesting an independent action of the otherwise suppressed A gene or its functioning up to a precursor stage. The basal part of the feather is possibly most suggestive of G in color, A in pattern and P in the rachis character. The barbs show a crest and a translucence scarcely present at all in P, but, like P, they do have barbules throughout their entire extent, except that in an occasional one the terminal fraction of a millimeter is free and pointed. These barbules, although present, are fewer and weaker than in P, giving the feather a loose, less compact, appearance.

PCC. In morphology the crest is clearly of the G type, but imperfectly so. The longest feathers are slightly in excess of 40 mm. Their color is of about the same intensity as that of GA, but as if overlaid with a brown wash making the result almost exactly intermediate between PC and GA, much brighter than the former, much duller than the latter. Proximally the barbules have a full complement of barbicels, but more distally the latter disappear and the barbules themselves become fewer and weaker. Instead of becoming iridophores as in P, many of the more distal ones appear merely as feeble rudiments, but they disappear completely only close to the tips of the barbs. It may be noted in passing that P, PC, PCC and C provide a transition from a condition in which color effects are mediated almost wholly through the iridophores to one in which iridophores are lacking and color is expressed through pigments in the barbs themselves.

PCCC. There is more diversity in this and in subsequent generations. The crest is copious and, in later generations, ranges in color from a red about equal to that of A down to a light cream. In PCCCC, two cousins, and many in later generations, have crests that would pass as pure G. On the structural side, early differentiation of the barbal crests, an enhanced terminal brush, and the distribution of barbules proved to be points of chief interest. These traits range from the configuration noted in PCC to typical G. Males with relatively high barbule counts show offspring with similar, medium, or low counts. One bird with hybrid ancestry on both sides shows an appreciably greater number and more extensive distribution than either his father or maternal grandfather. Since all blood other than hybrid was from G, in which this character is highly restricted, this return to a condition found in earlier generations, but still not equal to PC represents a recombination of at least two different P genes that had been perpetuated in different hybrid lines, or the establishment of homozygosity for one of them which even then was not adequate by itself to reproduce the full P condition.

Comment. It may be noted that in the crest, as elsewhere in the plumage, while grades of dominance and recessiveness are clearly indicated, the "recessive" gene usually makes its presence felt to a limited extent. This makes it the more difficult to distinguish between allelic and complementary genes but does not preclude detecting genetic difference between the species.

On grounds of morphology and the genetic evidence that has been presented it may be inferred that the genus *Chrysolophus* attained its more advanced specialization in respect to the crest through the effect of one, or possibly several, recessive mutations which have contributed to the differentiation of this genus from *Phasianus* or from a common ancestor of them both. On the other hand it would appear that *pictus* advanced over *amherstiae* as the result of a dominant gene which extends the already developed parietal crest anteriorly over the crown and forehead. While the latter gene is dominant in a C complex, it is recessive to what must be presumed to be its allele in P, indicating that despite the findings of Serebrowsky ('29) in chicken-pheasant hybrids, dominance need not necessarily

be an intrinsic property of the gene. It is particularly interesting that feathers which in A and P are morphologically very similar display opposite genetic behavior, showing that morphological similarity cannot be taken at its apparent face value without adequate genetic checks. Taking all three species into account, the behavior of these morphological traits requires that, as a minimum, two sets of alleles be postulated, one of these with three members.

In color the crest shows a wide range with the individual specimens falling into possibly nine categories, too many for a set of three alleles, but not for three independent pairs. While the available numbers were statistically inadequate, no case was found which on the basis of inspection, ancestry or progeny was inconsistent with the assumption that the color formulas for the three parent species could be represented respectively as PPaagg, ppAAgg, and ppaaGG. Such a scheme leads to the expectation of a triple recessive, ppaagg, and it is presumed that the "creams" which occurred in later hybrid generations are such. This coloring is sometimes encountered in G specimens in aviaries, where it may be attributable to mutation or possible earlier hybridization with A.

For expression of barbs and barbules, there must be at least two, and probably three genes to account for the brush tip and clubbed tip, both recessive in C; a pair of blending, or C-dominant genes for barbal crests; a somewhat recessive gene for the P, and perhaps A type of iridophore; at least two apparently non-allelic dominant and cumulative P genes for barbules and a C gene, possibly not allelic to either of these for free barbs. In addition, there can scarcely be less than three pairs of genes involved in differences in undercolor and length of afterplume. All told, this makes a minimum of fourteen chromosomal loci which influence the crest in these hybrids. Unless several of them are represented by multiple alleles, the number must be considerably greater. Most of the heterozygous manifestations are somewhat blending, but each species shows a considerable degree of dominance in some of its traits.

Cape

The cape may be defined as composed of those feathers which arise from the nape, approximately the region between the parieto-occipital suture and the upper border of the fifth cervical vertebra. In P there is little to justify setting this off as a special area. The feathers grade evenly into the plumage of the sides and lower part of the neck without sharp interruption in form or pattern. Most of them are about 20 mm long, with a good basal web, a distal third bearing iridophores of some 20 cells each, and a slight terminal fringe or brush. Those near the midline give a greenish, the more lateral ones a blue or purple, reflection.

C (*A* and *G*). The cape is one of the most characteristic features in *Chrysolophus*. Except for a few transitional feathers in front it is quite sharply defined and consists of some 200 or more feathers arranged in a dozen to fifteen rows. These feathers are under voluntary control to the extent that in the well-known display maneuver they can be spread fan-like in a large arc, or nearly complete circle in which the eye is almost at the center. Each cape feather is characterized by a terminal black bar followed by a white (A) or yellow (G) *display* zone, in most feathers another black bar, and then a long basal concealed section. Superficially cape feathers of the two species might at first seem to differ in little except color, but there prove to be several distinctive differences which probably signify a long period of independent evolution.

In outline, cape feathers of G, with a length up to 80 mm, are wedge- or bell-shaped, flaring toward the end into a broad truncate and somewhat eccentric extremity. In A, comparable feathers have a length up to 100 mm and, from before back, have distal ends that range from sharply pointed, through rounded, to merely obtuse.

The distal bar extends across the whole width of the feather and consists of a heavy concentration of barbs with very numerous barbules of the iridophore type, and a terminal brush tip. In G the bar is narrow at the margin, wider (2 mm) at the center, and has a slightly purple sheen. In A it is slightly wider throughout, with a narrow crescent of reduced pigmentation along its proximal border and a greenish sheen.

In the display zone of G the barbs are completely without barbules except for a very few reduced ones close to the proximal and distal edges. As the barbs enter this zone they acquire a high crest on their dorsal surface, making their vertical diameter much greater than their width. These crests are visible under low magnification and can easily be felt by drawing the feather between thumb and finger. Since the barbs are rather firmly anchored proximally and distally, the display zone appears as a grating of parallel bars, translucent because of the open spaces between them. Their color is a rich orange-yellow, from which no C-red can be extracted. In A all the barbs have barbules throughout the whole area and the crests are lower than in G. The display zone is somewhat wider in A than it is in G.

In G the proximal black bar extends all the way across the feather, but tends to be wider at its medial end. It has only slight iridescence. In A the iridescence is green, the bar does not reach either edge of the feather, is not strictly parallel to the distal bar and is frequently broken into two or more spots. In the most anterior feathers it is entirely lacking.

The basal, concealed, part of the feather is its largest portion and is well webbed in both species. In G the color shows a rather uniform transition from a dark khaki brown bordering the proximal bar to a pale buff just above the fluff and short afterplume. In A the undercolor is pure white, except that the fluff and afterplume are gray.

GA. Three hybrids all have cape feathers that are very close to those of A in color, size and pattern, except that they are less pointed or rounded and, on direct comparison, prove to have a very slightly yellowish or dusky wash. In one bird the similarity to A amounts to almost complete identity, except for a slight broadening of the proximal bar and the merest hint of a brown wash. A second has this wash in a more intensified form, remotely suggesting G in the concealed area, and the third shows an almost imperceptible tint at the distal margin of the display zone. However, except on careful inspection, A appears to be almost completely dominant in all these respects, which is quite different from the situation in the crest.

GAG. The one specimen preserved for study has cape feathers which are almost perfectly G in size and outline, but the display zone is as wide as in A, with most of the barbs passing through it having normal to weak barbules throughout. A few have short free areas. The pattern is pretty much G, but the colors are lighter. Other birds described from life, but not preserved, are recorded as "Golden" but the specimens were not then being examined critically for finer points.

PC. The intergeneric hybrid appears on superficial examination to lack completely any trace of a cape. Nevertheless, the feathers of the nape are actually longer (up to 50 mm) but at the same time even narrower than those of P. A compact web extends out for four-fifths of the rachis, beyond which the barbs bear compact iridophores. These give a violet sheen over a rich chestnut background, a color not approached by any of the parent species. The iridescent zone is followed by a narrow black and a broader rufous bar, suggesting, but by no means equalling, a P pattern and, at the same time, having a remote resemblance to a C cape pattern in miniature. The rest of the concealed portion grades in color from a dark to a very light brown as it approaches the dark fluff, a feature remi-

niscent of G coloring in this region. No trace of C morphology could be detected in any of the finer details.

PCC. In the first back cross the C cape reappeared, with feathers 75 mm long and roughly G in form. There is a terminal black bar, a light display zone, a faint proximal bar and a rather dark gray concealed portion leading back to a still darker fluff and afterplume. There is a dark green iridescence of the terminal bar and a light green on the proximal one. Almost the entire extent of the concealed portion also shows a green iridescence, a new feature, presumably an extension of the green iridescence in A. The display zone is fully as wide as in A and the barbs crossing it are lightly crested and have a full complement of barbules. The general effect is of an A feather reduced in size and dipped in a gray wash.

PCCC. In the second back cross generation, three specimens were studied and several others were recorded as superficially similar. In all of them the cape feathers are much as in the preceding generation, 65–80 mm long, and essentially G in outline, but with some other advances toward the G type. The two transverse bars are more distinct and the display zones between them are more sharply defined, with barbs showing a distinct dorsal crest. The central ones have barbules throughout their whole extent, dark in one, light (like A) in the other two. Some of the more lateral barbs are free for short distances (a G influence). The over-all color of the display zone while apparently white shows, on direct comparison with A, a faintly yellow tinge. In one, there is a distinct transverse brown bar appearing just below the proximal black one. Otherwise the color of the concealed portion in all of them is that of PCC, but diluted with a faint admixture of brown.

Later generations. The characteristics of the three PCCC birds were in a measure carried over to their descendants. For example, a son and grandson of the male with the concealed transverse brown band also showed the same color in broken pattern in their cape feathers. This and one of the others proved to be heterozygous for yellow since some of their immediate offspring showed definite yellow coloring in the display zone, and among them are a few almost perfect G specimens, in so far as the cape alone is concerned. In various birds of later generations yellow of the display zone appears in at least four tones. In intersex females a mixed pattern with a red component also appears.

Comment. So far as the present evidence goes, the occurrence of the highly distinctive *Chrysolophus* cape could be attributed to a difference between P and C of one main and only a few reinforcing genes. In the intergeneric cross the cape is almost completely obliterated, in the first back cross to C it reappears in clearly recognizable form and in the second back cross it attains almost full status. The chances are remote that the observed results would have been obtained if any considerable number of divergent gene pairs were involved. Aside from the genes controlling the main form of the barbal crest, there are apparently at least two pairs each for color and for pattern, and not less than two pairs regulating the four or more grades of barbulization. In addition to these there are obviously a number of minor modifiers with only moderate effects.

It would seem from this evidence that the initial advance of C over P, or their common ancestor, was mediated through a single recessive mutation which, when homozygous, is adequate to condition a well developed cape. Which, if any, of the other genes whose effects are superimposed on this were present in C before the main mutation, and which have resulted from subsequent mutations is not apparent; but within the framework for an organized C cape it would appear that G has made at least two significant advances over A: the more specialized form of the feather as a whole, and the highly

specialized free barbs of the display zone. The former is more or less recessive, the latter definitely so. In brief, it seems that with respect to the nuchal plumage G is the most highly specialized of the three species and that its main characteristics are due to three principal genes all of which are somewhat recessive. It shares one of them with A, none with P.

Neck ring

In the form of P considered here the neck ring is in effect two lateral white spots which are confluent behind, but not in front. Feathers for some distance below the ring continue to show some white in their undercolor, either as spots or stripes along the rachis. The ring is not represented in C where the homologous feathers are small, undifferentiated, and overlain by the long cape feathers. In the intergeneric hybrid the ring as such is completely suppressed, but in regions immediately beyond its normal site there is in the undercolor much concealed white which is not only more in amount, but extends further back than in P itself. There is no trace of this in PCC nor in subsequent back-crossed generations. Neither A nor G shows white in this region, nor do hybrids with the hypothetical formula P 66 : G 59 : A 3, from which it may be inferred that the white in PC is an emergent effect resulting from some synergistic action of P and A genes.

Mantle

Feathers comprising the mantle, about 130 in number, arise in the dorsal pteryla from the midneck region back to the level of a line between the posterior axillary folds. In P they are slightly notched at the tip and have a complicated pattern of iridescent blacks and browns. In C they have a wide convex distal margin and a color which is green in both species, but with a superimposed violet sheen in G. There is a heavy terminal brush border in both species, but the most characteristic feature is a subterminal transverse bar in which the iridophores are somewhat reduced in size and turned on edge, giving an effect not unlike that of the display zone in G but produced in an entirely different way. When the main part of the feather appears green, this line appears black, and vice versa. The width of the bar and border is about 2½ times as great in A as in G. In GA the feathers are intermediate in form, size, and structure of the modified area. In one GAG they were similar to GA. In PC the feathers are "normal" without the morphological specialization of either parent genus, except at the extreme caudal limit of the area where they become notched, suggesting a P influence. The color is somewhat suggestive of P, with no suggestion of C. In PCC they are G in outline, A in structure, but with the transverse bar even wider than in A. The color is rather nondescript with effects of both P and C genes in evidence. Among 21 specimens representing generations beyond the fourth, terminal parts of the feather were recorded as A in 2, G in 3, GA in 7, and intermediate between the latter two in 9. One bird with hybrid ancestry on both sides showed GA morphology but a new color pattern with yellow.

In the mantle region there appears to be a greater tendency than in the preceding region for genes from different sources to make their presence felt simultaneously, perhaps because feathers here are on the whole less specialized. The only highly specialized feature, the modified zone in C, is completely recessive in F_1 and when it does reappear in the first back cross it is in a modified, somewhat exaggerated form. In this and in many specimens in subsequent generations, there is good evidence of a reinforcing gene from P in which the trait itself does not occur. There must be at least 2 pairs of genes separating A and G, and others differentiating P and C.

Dorso-lumbar region. Rump

The 280 or more feathers of this region take origin in the dorsal pteryla from its

narrowest point, a virtual aptera between the shoulders, back to the oil gland on the upper surface of the tail. A continuous gradient in the size, form and pattern of these feathers prevents any satisfactory subdivision of the region. In many ways these feathers are the most diversified and interesting in the whole plumage, but consideration of space does not permit of their detailed treatment at this time. In P, from before backward, they range from 60 to 120 mm or more in length, from normal to notched to "dissected" in contour, and from a well-defined pattern to a complete disintegrated one. In A the lengths range from 50 to 75 mm, and in G from 65 to 90 mm. The distal end of the feather is truncated or notched in A, pointed in G. In both A and G there is a terminal, or subterminal, zone in which the barbs are free of barbules and crested as in the pileum. This specialization forms a band about 4 mm wide in A, 6 mm in G. Tips of the barbs prevailingly have pointed or brush tips in G, clubbed tips in A. In color, A is a clear lemon yellow in front and scarlet behind where the feathers overlap the base of the tail. In G the color is uniform throughout and about intermediate in tone between the crest and the display area of the cape.

In these three species, as in many others, there are specializations which tend to produce the optical effect of a fringe on the sides of the rump. This effect is attained in such a diversity of ways that one might infer that it must have some special value to the bird. In A it is only slightly marked and feathers differ but little in form from the normal contour feathers of, for example, *Syrmaticus reevesii*. The web, however, is not entirely intact, even proximal to its margin of free barb tips, but is broken into segments by the separation of barbs into groups of irregular size, as if the barbs were too many or too weak to hold together. In G where the barbs are longer and the feather more pointed this grouping is much more pronounced and the fringed effect thereby considerably enhanced. In P there is the same tendency for the barbs of each feather to break up into small aggregates, but the fringing effect is even more pronounced because these barbs are longer and the splits between them extend more nearly to the rachis. In addition, two other factors accentuate the effect. While in P the barbs have no free areas as in C, their barbules nevertheless are short and closely appressed, giving each barb (under magnification) a kind of rat-tail appearance. The gross effect is superficially suggestive of the fringe on the rump of the domestic fowl, although morphologically the two are quite different. Finally, the fringing is still further enhanced by the rather bizarre morphology of the barbs themselves, among which there are otherwise normal ones that are only a fraction as long as their neighbors. These short barbs, of variable length, are distributed irregularly through the distal part of the feather. An occasional one bifurcates and not infrequently there are cross-anastomoses between them, as if the species were still in an evolutionarily unstable transitional stage.

In the inter-generic hybrid, PC, the largest feathers attain a length of about 100 mm. In color they are a dark rufous with a coppery sheen. Copious barbules extend to the tips of all barbs, giving the feathers a firmer appearance than in any of the ancestral species. There is neither the dissected appearance of P and G nor the free tips of G and A. Thus in the first generation hybrid all the distinctive specializations in color, pattern and structure are lost, while in later hybrid generations there is a considerable diversity, with various recombinations of traits, and a few recurrences of lost traits in offspring of pairs in which both parents had hybrid ancestry. No less than 14 sets of genes, many of them involving multiple alleles, must be postulated to explain the genetic behavior of the plumage traits in this region.

Tail

There are 8 or 9 rectrices on each side of the tail with gradations in length from the central which is longest to the most lateral which is shortest. Immediately in front of each rectrix, except the two central ones, is a smaller feather, here called the true covert. The central rectrices are in line with these coverts, to which they may be morphologically related.

In P the central rectrix is flat, so that when laid on a table it makes contact all along without forming a transverse or longitudinal arch. It reaches a maximum length of about 540 mm and a width of about 50 mm. The ground color is a gray-green khaki crossed by about 25 transverse bars, deep rufous laterally and black medially with an offset at the rachis. In C the corresponding feather is strongly vaulted with the margins drawn in on both sides, and it is also arched longitudinally. Throughout most of its extent the feather of one side embraces that of the other within its concavity. In A the length exceeds 720 mm, with a width of 90 mm; in G the corresponding figures are 560 and 55. In A the ground color is white crossed by about 25 crescentic black bars between which there are numerous irregular black markings. In G the ground color is dull brown broken up by a flat black reticulum into spots of from 2 to 5 mm in diameter.

Among the pure species and hybrids the central tail feathers appear in four forms: flat in P, slightly curved in PC, definitely vaulted in PCC, and strongly arched in PCCC and later generations. Apparently each genus has two complementary genes affecting the shape of these feathers. In total length the tail of A is much longer than in either of the others and in interspecific and intergeneric crosses this advanced character appears as a clear-cut simple dominant. This is in contrast to the very long tail of Reeves pheasant which is recessive to that of P. In ground color AG is intermediate between A and G and the pattern is disintegrated, while PC has a rich uniform brown color without pattern. In one line the appearance of a brown central rectrix continued with little change down to PCCCC. In these central rectrices, where individual feathers can be homologized, even between genera, specializations in gross structure are more pronounced in C, with finer details more apparent in P. Both sets of specializations are suppressed in the first generation hybrid.

The longest tail covert, that just lateral to the central rectrix, has the following measurements in the three species: P, 105×30; A, 240×30; G, 240×25, all in mm. In C there is a constriction in the distal part of the feather followed by a scarlet or red tip. This tip is 90×16 mm and blunt in A; 100×10 mm and pointed in G. Both contain C-red, but titration shows the intensity to be about $2\frac{1}{2}$ times as great in G as in A. In GA the morphology is essentially G, the color intermediate, and the pattern of the basal part essentially G. In PC the feather is somewhat longer than in P, with no constriction, but with a pointed tip as in G, and a brown color. PCC shows a feather 120×28 mm, light brown distally, with barbules throughout, in contrast to C where they are lacking distally. Some specimens in the PCCC generation come very close to being typically G in respect to their tail coverts.

Other areas

Discussion of other tail feathers and those of the wings may be omitted. As to feathers of the under parts, it may be noted only that in GA there is some irregularity of dominance in the throat and upper breast areas where A characters are more or less apparent in both structure and color. But for the greater part of this region the red of G is almost completely dominant except in one particular. At the level on the breast where black and white areas meet in A, and where the color is uniformly red (with dark undercolor) in G, there is consistently on each

side a conspicuous yellow spot which tends to extend across the breast nearly meeting its fellow of the opposite side. This entirely new trait, not present in either parent species, carries over into some GAG specimens. In the PC hybrid the under parts are rufous in tone, like much of the rest of the plumage, with neither the A pattern nor the GA markings showing. In PCC the A pattern is vaguely indicated but not the AG mark, which however does reappear in some individuals of later generations.

Discussion

The observed differences in plumage traits find a plausible explanation on the assumption that they are regulated by determining genes whose alleles are variously distributed among the three species studied. This is particularly true when the semi-autonomous plumage regions are studied one at a time. In each region progressive back-crossing is followed for the most part by a rapid return to the characters of the form (G) used as the pure species, and this seems to be the case also with such of Ghigi's ('37) experiments as can be compared with the present ones. Anderson ('49) has pointed out that in general something of the sort is to be expected in this kind of introgressive hybridization. On the other hand, when minor traits and different regions are taken into account the results appear much less uniform and a considerable degree of mosaicism becomes apparent. There has not yet been time to determine definitely whether or not any of the new combinations can be fixed in a stable breed, or "incipient species."

A number of hybrid characters appear as if new, being unrepresented in the parent species. Conspicuous among these are the over-all brown color in PC and the yellow breast mark in GA. Darwin would presumably have suspected these of being traits submerged in the process of speciation and again released by hybridization. The fact that most of the more striking specific characters appear as if recessive would add some plausibility to such an assumption were it not that the galaxy of modifying and supporting factors is too great to admit of any such simple analysis as Darwin himself attempted. The modifiers, which for the most part become genes in their own right as the examination is focused on finer and finer details, would seem to be extremely important elements in the determination of specific characteristics and may furnish a basis for the amazing stability encountered in many seemingly trivial characters of wild species.

It is these associated genes, polygenes, that make it especially difficult to recognize allelic differences between species. Indeed, it would seem that one of the main differences between domestic breeds and wild species is in the relative numbers of minor accessory genes that provide a kind of milieu in which the major genes find expression. If by chance a considerable number of minor genes of one species are recessive, or even blending, with reference to those of another species, hybridization might give a false impression of recessiveness in a major gene associated with them. In these studies the form of the pileum of A appears to be recessive to that of P, but according to Huxley ('41) in a hybrid between A and *Lophophorus impeyanus* the A type of crest appeared to be dominant. It has just been noted that the relative shortness of tail in P is dominant to the great length of *Syrmaticus reevesii* but recessive to the moderate length of A. It would require extensive genetic tests to determine the true relation of these separate alleles to each other.

The effect of different regions of the body on the expression of individual genes proves to be another complicating factor. In C, but not in P, certain feathers show a striking specialization which involves loss of barbules over a considerable extent of the barbs, with the concomitant development of a high, translucent cornified barbal crest. In both A and G this specialization finds expression

in feathers of the pileum, of the rump and tips of the upper tail coverts. In G it is also seen in the display region of the cape, but not so in A. Both species have the genetic background to permit this high grade of specialization, but there are thus different regional limitations on its expression.

A similar phenomenon is encountered in the distribution of C-red in the plumage. As already noted, it or its immediate precursor is not synthesized at all by P. In G it is produced abundantly in the breast feathers and appendages of tail coverts, but not in feathers of the crest or rump. In A what is almost certainly identical material is developed with maximum intensity in the pileum, is present in feathers of the rump and, somewhat diluted, in the tail coverts, but it is completely absent from the breast. It would not seem likely that there are separate genes for the production of this pigment in each region where it occurs, but rather that, given the gene in the first place, the several regions of the body are so conditioned by other genes which differ between the species that they severally do, or do not, favor realization of an innate capacity to elaborate the substance.

In this connection it may be recalled that all the characters discussed in this paper are immediately dependent on the biochemical environment in which each individual feather develops. A few milligrams of estrogen, or even diethylstilbestrol, properly administered, serve to inhibit development of all the special structural and pigmentary characteristics that have been discussed and, conversely, removal of certain hormones from the female results in the appearance of such traits as a white neck ring, or red tail coverts in birds where these characters normally would never occur. Such facts do not in any way detract from the significance of the observations but, on the contrary, serve to emphasize the validity of the genetic factors as well as their dependence on just the proper genetic and biochemical milieu if they are to manifest themselves at all.

In the matter of speciation the *tenebrosus* trait may be worthy of passing consideration. This trait is clearly conditioned by a single gene whose primary effect is to cause a general increase in melanin production, but with results which are by no means the same in different parts of the plumage, since there are regional differences in intensity of pigmentation and pattern. These differences are no doubt chiefly due to genes that were already present before the *tenebrosus* mutation occurred, but it would seem not impossible that around this major mutation a topographically even more diversified pattern might ultimately become established through accumulation of further mutations in minor genes. In the case of A and G the plumage also differs throughout its whole extent, but if this is due to an original major mutation with a generalized effect, the universality of that effect is now masked by the superimposed action of mutant genes whose influence is more localized. In any event, and however they may have arisen in individual cases, it seems clear that among the species studied there are a few rather simple major differences which are supported and reinforced by the effects of a large number of lesser genes.

There is an alternative possibility which these data do not disprove. Instead of most traits having been initiated by a significant major mutation whose effect was immediately conditioned by such genes as were already present, and subsequently reinforced or modified by new mutations, it might be that prevailingly traits have developed more nearly in a Darwinian sense through small, essentially non-specific, mutations which have gradually built up something whose ultimate character was in no sense foreshadowed during its early stages. The following consideration may be pertinent in this connection.

After hybridization, followed by successive back-crossing to one parent form, the statistical expectation that a simple recessive trait will reappear in an individual from the first, second, third, or later back-

cross is 1/2, 3/4, 7/8, 15/16 . . . , but if the trait is dependent on two or more recessive alleles the corresponding expectations are indicated by the appropriate fractions raised to the *n*th power, where *n* indicates the number of independent recessive genes that are involved. Thus if there are three recessive genes, all necessary for the appearance of a trait, it would be expected to reappear in PCCC, for example, in $(3/4)^3$, or about 42% of the individuals, whereas if the determination were due to a single major gene whose effect is merely modified by other genes, it should occur in recognizable form with nearly twice that frequency (75%). Most of these data, and those from other studies on pheasants, seem more in accord with the latter alternative, but obviously they are not adequate to be decisive. The question still remains as to whether traits of diagnostic significance have crystallized from aggregates of independent random mutations or have been based prevailingly on the effect of a more or less major mutation which has served as an evolutionary point of departure or "nucleus" for further specialization.

Summary

The discussion has centered around genetic, and presumably phylogenetic, relations of gross and microscopic features in the male plumage of four types of pheasants, their hybrids and members of successively backcrossed generations. The forms used were *Phasianus colchicus mongolicus, P. c. tenebrosus, Chrysolophus pictus,* and *C. amherstiae.* Feathers from each of the principal regions of the body were examined microscopically for finer details and the behavior of recognizable traits studied in the hybrids. As a basis for interpretation it has been assumed that all of the species have chromosomal loci which are represented in each of the others, and that the species used probably had a common ancestry at a phylogenetically not very remote date.

Most of the traits which might be considered as diagnostic in the systematist's sense, prove to be dependent for their full expression on a relatively large complex of separately heritable units. These units are not of equal value so far as the main trait is concerned but usually consist of one, or at most a very few major elements that are reinforced and supported by more numerous minor ones. The situation is well illustrated by the features which give character to the capes of *pictus* and *amherstiae,* superficially simple but actually quite complex. While it is to be expected that in any series of mutations there will be a considerable range in potency, the number of cases in which the evidence points to one major gene and numerous subsidiary ones is great enough to suggest that the evolution of many of these plumage traits may have been effected through the initial appearance of a mutation of sufficient magnitude to produce a very appreciable effect from the beginning, and that this effect has been reinforced and modified by genes whose influences are individually less direct or less powerful.

Many of the lesser factors are so restricted or so localized in their action that separate regions of the plumage come to behave almost as autonomous units. A localized regional influence is possibly indicated in the plumage of the forehead in *mongolicus* and *amherstiae* where close similarity results from diverse genetic backgrounds. Such a form as the melanotic *tenebrosus* suggests the possibility that extensive differences, even in pattern, may have been brought about by a single major mutation which from the first might automatically show different effects in different regions. In the differentiation of *Phasianus* and *Chrysolophus* several such characters seem to have appeared, among them two striking acquisitions by *Chrysolophus*—(a) development of an ornamental barbal crest with concomitant loss of barbules, and (b) production of the pigmentary precursor which is here called C-red. Each of these characters varies with the region in which it appears in *Chrysolophus,* and is entirely lacking in *Phasianus* (which, incidentally, "imitates" both of them). With reference to these

two traits the differences between the two genera are qualitative, while those between the two species of *Chrysolophus* in which both of them occur may more properly be designated as quantitative, due merely to the effects of modifying genes.

If it be assumed that, in contrast to the more generalized traits, specialized diagnostic features are phylogenetically the more advanced, it would then appear that more frequently than might have been expected evolutionary progress in plumage differentiation has been effected through the appearance of recessive mutations. Evidence bearing on this question is difficult to assess since the picture is generally obscured by the secondary effects of modifying genes and since most of the more conspicuous traits have proved to be somewhat "blending" in the sense that they are neither completely dominant nor wholly recessive. One conspicuous exception is in the length of tail in *amherstiae,* which seems to be completely dominant over that of *mongolicus* and *pictus.* There are various minor characters which likewise seem to be fully dominant, but for the most part the major diagnostic traits behave as if they were primarily recessive to the alternative conditions. This is true, for example, of the *Chrysolophus* cape, crest and pigment precursor, and of the *Phasianus* neck-ring, color pattern and specializations in feathers of the rump. In short, the more highly distinctive generic and specific traits in each of the species tend to become submerged in the presence of a foreign genome. That they tend to revert to an ancestral form is open to question.

It is of interest from a phylogenetic point of view that *Phasianus* and *Chrysolophus* as genera are distinguished by many plumage traits which seem to be conditioned by groups of primary and secondary genes which, as aggregates, behave in first generation hybrids very much as if they were simple, recessive units. This may mean that recessive secondary genes have an advantage over dominant ones since recessive homozygosity would seem to be more easily attained and, once attained, potentially more stable. Within the genus *Chrysolophus,* however, this tendency is less marked and, unless it be assumed that one species has undergone retrograde evolution, it appears that *pictus* and *amherstiae* have accomplished their mutual differentiation through the acquisition of both dominant and recessive traits.

Literature Cited

Anderson, Edgar. 1949. Introgressive hybridization. New York, John Wiley and Sons, ix, 109 pp.

Beebe, William. 1926. Pheasants, their lives and homes. New York, Doubleday, Page and Co., vol. II, p. 110.

Bohren, B. B., R. M. Conrad and D. C. Warren. 1943. A chemical and histological study of the feather pigments of the domestic fowl. Amer. Nat., **77**: 481–518.

Bruckner, J. H. 1939. Inheritance of melanism in pheasants. J. Hered., **30**: 45–52.

Danforth, C. H. 1942. Sex inversion and the plumage of birds. Biol. Symposia, **9**: 67–80.

Danforth, C. H., and Gunnar Sandnes. 1939. Behavior of genes in intergeneric crosses. J. Hered., **30**: 537–542.

Ghigi, Alessandro. 1937. Nuovi fatti di sterilità e di intersessualità da ibridazione. Rend. R. Accad. Sci. Ist. Bologna, 1936–1937: 1–7.

Haig Thomas, Rose. 1914. The transmission of secondary sexual characters in pheasants. J. Genet., **3**: 275–298.

Haig Thomas, Rose. 1916. Color and pattern transference in pheasant crosses. J. Genet., **5**: 243–280.

Huxley, Julian S. 1941. Genetic interaction in a hybrid pheasant. Proc. Zool. Soc. Lon., **111A**: 41–43.

Nitzsch-Burmeister. 1840. System der Pterylographie von C. L. Nitzsch nach seinen handschriftlich aufbewahrten Untersuchungen verfasst von Herman Burmeister. Halle. 228 pp.

Phillips, John C. 1921. A further report on species crosses in birds. Genetics, **6**: 366–383.

Serebrovsky, A. S. 1929. Observation on interspecific hybrids of the fowl. J. Genet., **21**: 327–340.

Test, Frederick H. 1942. The nature of the red, yellow and orange pigments in woodpeckers of the genus Colaptes. Univ. California Pub. Zool., **46**: 371–390.

THE INFLUENCE OF LIGHT ON THE MATING BEHAVIOR OF SEVEN SPECIES OF THE *DROSOPHILA MELANOGASTER* SPECIES GROUP

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INTRODUCTION

Philip, Rendel, Spurway and Haldane (1944) and Rendel (1945) found that *Drosophila subobscura* Collins cultures could not be maintained in a dark place because matings did not occur in the species in the absence of light. Wallace and Dobzhansky (1946) investigated the mating behavior of *D. subobscura*, *D. pseudoobscura* Frolova and *D. persimilis* Dobzhansky and Epling in the light, in total darkness, and in red light. Although *D. persimilis* and *D. pseudoobscura* were found to court freely in all three situations, they confirmed the previous findings that *D. subobscura* would not copulate in the dark, nor did the males even court in the red light. *D. subobscura* females, however, were inseminated by both *D. persimilis* and *D. pseudoobscura* males when isolated with them in the dark. The reciprocal crosses never gave any inseminations and direct observation in red light showed that the *D. subobscura* males never courted.

Previous to the publication of Wallace and Dobzhansky's findings, one of us (T. C. H.), working with Mr. T. T. Liu at Chekiang University, Hangchow, China, discovered that *Drosophila auraria* Peng, a member of the melanogaster species group, would not copulate in the absence of light but would do so at a much reduced rate in red light. These findings were never published and the original data are now unavailable.

Recently, while making a detailed study of the mating behavior of various species of the genus *Drosophila*, one of us (H. T. S.) ascertained that *D. auraria* and its close relatives, *D. rufa* de Meijere and *D. montium* Kikhawa and Peng, all had basically similar mating behaviors. In addition to these three species, the mating behaviors of four other species of the *melanogaster* species group, i.e., *D. melanogaster* Meigen, *D. simulans* Sturtevant, *D. takahashii* Sturtevant, and *D. ananassae* Doleschall, were studied. The basic mating behavior of the first three of these species is essentially similar but it is different from that of *D. auraria* and its close relatives, while *D. ananassae* appears to be intermediate between the *auraria* type of mating behavior and the *melanogaster* type. It has been known since Payne's studies (1910) that *D. melanogaster* can reproduce successfully in the dark. Thus, it seemed interesting to determine whether or not ability to mate in the dark is correlated with a particular type of mating behavior.

MATERIALS AND METHODS

A single strain of flies for each of the seven species has been used, with the exception of *D. simulans* for which two strains were employed. The University of Texas collection numbers and the point of geographical origin of each of these strains are as follow:

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D. auraria	1736.1	Hangchow, China
D. rufa	1736.3	Hangchow, China
D. montium	1736.4	Hangchow, China
D. ananassae	228.4a	Baton Rouge, Louisiana
D. takahashii	1736.2	Hangchow, China
D. simulans	1339.1a	Guerreno, Mexico
D. simulans	1976.2a	Ennis, Texas
D. melanogaster	Stephenville +, Stephenville, Texas	

All experiments were conducted in an air conditioned laboratory where the temperature is maintained at 72° ± 1° F. Specimens were removed as virgins from 100 mm. × 33 mm. culture vials, placed in glass isolation vials of the same size with food, and aged for four days. On the fifth day after emergence, the flies were etherized and ten females and five males were placed in 100 mm. × 33 mm. glass vials containing fresh food. Half of the vials were placed in light-tight cardboard containers and the other half in an open metal tray; all were then placed on an open shelf in the laboratory. The males and females were exposed to each other under these conditions for periods of one day, two days, and seven days, except for a few instances in which the period was extended to eight and ten days. At the end of the exposure time the specimens were etherized, the females sorted out and dissected, and their seminal receptacles and ventral receptacles examined for the presence of sperm. The food in the isolation vials was also checked for presence of larvae.

These procedures gave conclusive results for all species except *D. rufa*, *D. takahashii* and *D. simulans* 1339.1a. Data from these three were erratic and inconclusive regardless of whether the individuals had been kept in the light or the dark situations. In the case of *D. simulans*, stock 1976.2a which had been recently established in the laboratory from a wild strain was used to replace stock 1339.1a. No other strains of *D. rufa* and *D. takahashii* were available, so the following two methods were employed: (1) Small mass cultures, consisting of four day old virgins and utilizing the same sized vials as above, were made up and these were then handled in a manner parallel to the 10:5 matings; (2) small mass cultures were made up, using freshly emerged adults, and these were then checked for inseminations three to seven days after introduction.

Mating Behavior of the Species

These species all belong to the *melanogaster* group of the subgenus *Sophophora*. *D. subobscura* and its relatives belong to the *obscura* group of the same subgenus and the two groups are closely related. No two of these seven species have identical mating behaviors when the entire sequence of courtship and copulation is observed, although *D. melanogaster* and *D. simulans* are similar in all observable aspects except in one detail. The basic pattern of all seven, however, falls into two major types with *D. ananassae* in an intermediate position. The specific behavior of *D. auraria* and *D. melanogaster* will illustrate these two types.

When virgin, sexually mature males and females of *D. auraria* are placed together, the male exhibits the usual male drosophilid behavior of investigating other individuals by approaching and tapping with his fore legs. If a female has been tapped, the male circles to the rear and, facing the female's posterior, orients himself carefully until he is standing several millimeters away from the female and directly behind her. The male then crouches slightly, curls the tip of his abdomen under, and makes a running lunge at the female, propelling his body under her wings and grasping her abdomen with his legs, especially the fore legs. The male tarsi bear on the two basal joints heavy sex combs, each with ten or more teeth whose axes are at right angles to those of the tarsal joints, with the tips pointing inward. Further, the tarsal segments are so formed that the tarsus is concave on its inner surface, thus giving a bow-legged appearance to the males. The fore tarsi appear primarily responsible for grasping the female. Having

mounted and grasped the female, the male now proceeds to extend one wing to 90° and vibrates it, at the same time attempting by means of abdominal movements to insert his phallus into the female genitalia. A receptive female droops and extends the abdomen, spreads the genital plates, and allows the male to achieve insertion. Copulation then proceeds with the male retaining his grasping position without any modification, and averages 6′37″. If the female is non-receptive she has several ways of successfully resisting:

(1) Commonly when the male starts to circle to the rear, the female turns or spins about so that she keeps herself *facing* the male. He, therefore, never has a chance to attempt to mount.

(2) She may spread her wings slightly and raise her abdomen high into the air, almost standing on her head, thus making it impossible for the male to mount. Females that are feeding or have recently been fecundated usually utilize this method.

(3) The female may decamp very rapidly.

(4) If the male succeeds in mounting, the non-receptive female kicks vigorously, vibrates both wings, shakes her entire body, and at the same time keeps the tip of her abdomen close to the substrate with genital plates closely appressed. The males invariably are dislodged in a short time.

Finally, it should be noted that after the males have tapped and started to court vigorously, they often then omit the tapping action. This corresponds closely to the conditions found in *D. subobscura* as recorded by Rendel (1945) and confirmed by our own observations.

D. rufa has a mating behavior similar to that of *D. auraria* except that one to two minutes after coitus has been achieved, the male releases his grasp upon the female's abdomen and pushes his way forward and upward, thus spreading the female's wings, and comes to stand upon the female's dorsum. In repelling, the females have not been observed to elevate the abdomen as do *D. auraria* females but they are able effectively to repel the males by fluttering the wings. The average duration of coitus is 20′14″.

D. montium is also similar to *D. auraria* in mating behavior except that, after the male has mounted, the receptive female spreads her wings when the male vibrates. If the female remains quiet during the copulation, the male releases his grasp with his fore legs and stands upon the female. If she becomes restless he resumes his grasp. The female uses the same manner of repelling the males as does the *D. auraria* female. Also, *D. montium* males circle non-receptive females, raising and lowering their wings as they move about the females. The average copulation time is 3′46″.

A *D. melanogaster* male, when courting, first taps, then circles to the rear and takes up a position close behind the female. In this position, the male curls the tip of his abdomen under and forward, and simultaneously extends one wing to 90° and vibrates it intermittently, at the same time intermittently licking the female's genitalia with his extended proboscis. These actions may or may not be synchronous but always occur intermittently. Between the bursts of activity, both the proboscis and wings are returned to the normal resting position. The male also may tap with his fore legs while courting the female.

The receptive female apparently indicates acceptance of the male's overtures by spreading her genital plates apart. Thereupon the male brings the tip of his abdomen forward and against the female's genitalia and at the same time he lunges forward and upward, grasping the female and pushing her wings apart. Apparently his head and his fore and middle legs are used for this action. Having achieved coition, and having spread the wings, he then mounts still farther forward and grasps the dorsal surface of the basal area of the female's wings with his fore tarsi.

The non-receptive female repels by a variety of means, e.g.: (1) by fluttering

her wings, kicking with her hind legs and extruding; (2) by depressing the tip of the abdomen; (3) by decamping; and (4) if in the act of feeding, by elevating the tip of the abdomen and extruding.

Males repeatedly try to mount non-receptive females and just as often are dislodged. They try further to stimulate such females by circling them and then returning to the rear courting position. Observed copulations average 18'14".

The *D. simulans* mating behavior appears similar to that of *D. melanogaster* except that the male, in the act of circling the non-receptive female, often stops either directly in front of her or in front of one of her eyes and rapidly scissors both wings, gradually increasing the amplitude of the wings until they are extended 70° to 90° and then held briefly in this position. The wings then drop to normal position and the male continues to circle until he reaches the rear station. Furthermore, the female spreads her wings slightly (10° to 15°) when giving the acceptance response. Average copulation time is 16'52".

D. takahashii behaves similarly to *D. simulans*, except that when the male stops in front of the female when circling, he waves his wings outward and upward to 45° and holds them in this position very briefly. Copulation time averages 17'30".

D. ananassae is intermediate between *D. auraria* and *D. melanogaster* in many ways. The male taps, goes to the rear, curls the tip of the abdomen, and then lunges onto the female, grasping her abdomen. A receptive female spreads her wings and genital plates, allowing the male to achieve coitus. The male then mounts forward between her wings and holds on as does *D. melanogaster*. A non-receptive female kicks and curls down the tip of her abdomen, thus rendering coition impossible, and quickly dislodges the male. The male circles non-receptive females, scissoring both wings, and repeatedly tries to mount. Some females, especially those recently fecundated, elevate their abdomens and extrude when tapped by the males.

Wheeler (1949) and Hsu (1949) place *D. melanogaster* and *D. simulans* in the melanogaster sub-group. *D. takahashii* and *D. lutea* form the takahashii sub-group. *D. ananassae* plus *D. bipectinata* form the ananassae sub-group. *D. auraria*, *D. rufa* and *D. montium* belong to the montium sub-group. The seven species studied thus represent four sub-groups of the melanogaster species group.

Insemination Experiments

D. auraria, as shown in table 1, never copulates in the dark. Two hundred and eighty females were exposed to males under lightless conditions, and 270 of these were alive and utilized for dissection at the end of the exposure time. Not a single one of these females was fecundated. On the other hand, 71.54 per cent to 85.71 per cent were inseminated under illuminated conditions, the length of exposure gradually resulting in an increased percentage of fertilized females. Hsu, while at Hangchow, China, maintained small mass cultures of this species in total darkness for more than two weeks and never found a single female to be impregnated.

D. montium (see table 1) mates as well in the darkness as it does in the light. Both one and two day exposures gave a higher percentage of insemination in dark-exposed females. The χ^2 for one day is certainly not significant, but for two days it is. We have no explanation for this except that despite all precautions it is impossible to control rigidly all environmental conditions. The food may accidentally become contaminated with bacteria; the yeast may grow slowly; the volume of space above the food may vary, etc. Perhaps some one or several such factors may be responsible for the high number of unfecundated females in the two day illuminated experiments. Seven day exposure clearly shows that *D. montium* breeds freely in both the light and the dark under the conditions in which the experiments were conducted.

TABLE 1. *Insemination data on* D. montium *species sub-group*

	Age in days	Expos. period (in days)	Type mating	Light			Dark			χ^2
				No. ♀ intro.	No. ♀ dissec.	% fec.	No. ♀ intro.	No. ♀ dissec.	% fec.	
Auraria	4	1	10:5	130	123	71.54	30	27	0.00	
	4	2	10:5	100	95	80.00	70	66	0.00	
	4	7	10:5	60	56	85.71	180	177	0.00	
				290	274		280	270		
Montium	4	1	10:5	60	52	86.54	20	18	94.49	0.83
	4	2	10:5	30	28	67.86	70	59	89.83	6.43
	4	7	10:5	20	19	94.74	40	35	85.71	1.01
				110	99		130	112		
Rufa	4	1	10:5	10	9	11.11	—	—	—	
	4	2	10:5	100	99	7.07	10	9	0.00	
	4	7	10:5	110	77	10.39	90	60	3.33	2.48
	4	10	10:5	60	54	48.15	—	—	—	
	0	3–4	Mass	—	38	21.05	—	15	6.67	1.58
	0	5–6	Mass	—	33	63.64	—	42	7.14	27.10
	0	7	Mass	—	187	73.80	—	196	31.63	16.79
				280	497		100	322		

D. rufa (see table 1), when aged for four days before introduction, gave a low insemination rate in both light and darkness even when exposed for seven days. The χ^2 of 2.48 for seven day exposure shows that there is no significant difference between light and dark conditions. Ten day exposure under lighted conditions raised the percentages of fecundated individuals to 48.15 per cent. This failure of a large portion of the females to become inseminated during a short period of time when exposed to a limited number of males is apparently a common occurrence in many strains that have been kept under laboratory conditions for a period of time. Small mass matings were therefore resorted to and, instead of aging the specimens for four days, they were introduced into the experimental conditions on the day of emergence. Even under these conditions, the percentage of insemination was low except for the seven day lighted specimens. In three-four day exposures the percentage fecundated is 21 per cent in the light and 7 per cent in the dark. Because of the small number tested the true difference might be considerably greater. However, the χ^2 of 1.58 is not significant and hence there is no proof that any true difference is involved. There are other instances in this study where such reasoning can well be applied to the problem of evaluation of the χ^2s, but for the sake of brevity the matter is not again discussed in detail. For the five and six day experiments, higher χ^2s of 27.10 and 16.79, respectively, show significant differences. *D. rufa* apparently reaches copulatory receptivity on days three-four. In the small mass cultures of three-four days' age, one out of 15 females (6.67 per cent) kept in darkness was fecundated, while 8 out of 38 (21.05 per cent) kept in light were fecundated. The five-six day cultures, when compared to the three-four day cultures, showed a great increase in the percentage of females fecundated in the light (21.05 per cent to 63.64 per cent) but in the dark less than 1 per cent (0.57 per cent) increase was registered. Mass cultures

kept for seven days showed the percentage of fecundated individuals increased under both light and dark conditions, but there existed a great percentage difference between the two and the χ^2 is 16.79. Thus, *D. rufa* can and does mate in darkness but apparently matings are seriously interfered with by the absence of light.

D. ananassae (table 2) clearly mates as well in the dark as in the light. The χ^2 of 3.48 value for the seven day exposure is probably due to the fact that 14 of the females died during the darkness experiment, while only five were lost in the parallel lighted experiment. During a seven day period with only small numbers of individuals in the vials, the food surface often becomes sticky and individuals are trapped and killed. Fecundated females are probably more often trapped in this fashion during oviposition than are non-fecundated females, since fecundation greatly increases the number of eggs laid. Normally the insects, except when feeding or ovipositing, prefer to rest on the walls of the containers and thus fecundated females are exposed to entrapment much more than non-inseminated females.

D. melanogaster (table 2) mates readily in either the light or the dark, and the percentage of its inseminated individuals was found to be higher than in any other species. There is, however, a slight indication as shown by one day exposure that darkness has a slightly inhibiting effect upon copulation.

In *D. simulans*, unlike *D. melanogaster*, copulation is greatly inhibited by darkness (see table 2, stock 1976.2a). Although the χ^2 of the two day exposures is considerably lower than that of one day, it is still highly significant. However, additional exposure for seven days in the dark did not increase the percentage of insemination. In fact, in these particular experiments, it was only 2.75 per cent higher than resulted from one day exposures. *D. melanogaster* and *D. simulans* are unquestionably the two most closely related of any of the species utilized in these experiments, yet they show

TABLE 2. *Insemination data on* D. ananassae, D. melanogaster, *and* D. simulans

	Age in days	Expos. period (in days)	Type mating	Light			Dark			χ^2
				No. ♀ intro.	No. ♀ dissec.	% fec.	No. ♀ intro.	No. ♀ dissec.	% fec.	
Ananassae	4	1	10:5	80	74	18.92	80	74	16.22	1.28
	4	2	10:5	70	60	40.00	90	87	47.13	0.43
	4	7	10:5	100	95	81.05	110	96	68.75	3.48
				250	229		280	257		
Melanogaster	4	1	10:5	40	40	97.50	110	98	92.86	1.12
	4	2	10:5	20	20	100.00	70	64	96.86	0.64
				60	60		180	162		
Simulans	4	1	10:5	90	83	90.36	133	129	3.01	168.11
1976.2a	4	2	10:5	100	93	72.04	150	138	15.22	76.07
	4	7	10:5	—	—	—	100	87	5.75	
				190	176		383	354		
Simulans	4	1	10:5	20	20	10.00	20	18	0.00	
1339.1a	4	4	10:5	10	8	12.50	10	9	0.00	
	4	7	10:5	20	13	0.00	30	23	0.00	
				50	41		60	50		

TABLE 3. *Insemination data on* D. takahashii

	Age in days	Expos. period (in days)	Type mating	Light			Dark			χ^2
				No. ♀ intro.	No. ♀ dissec.	% fec.	No. ♀ intro.	No. ♀ dissec.	% fec.	
Takahashii	4	1	10:5	20	19	5.26	20	19	0.00	
	4	2	10:5	50	47	6.38	30	25	0.00	
	4	4–5	10:5	20	16	31.25	30	26	0.00	
	4	7–8	10:5	210	142	25.35	230	129	0.00	
	4	10	10:5	—	—	—	50	28	0.00	
	1	1	10:5	120	102	3.92	—	—	—	
	0	6	10:5	60	44	56.82	80	66	19.70	16.08
	4	1	Mass	—	45	24.44	—	—	—	
	4	2	Mass	—	81	17.28				
	4	6	Mass	—	—	—	—	95	7.37	
	0	5	Mass	—	39	66.67	—	33	0.00	34.43
	0	6	Mass	—	87	81.61	—	56	78.57	0.20
	0	7	Mass	—	28	57.14	—	45	64.44	0.37
					650			522		

great differences with respect to mating behavior in the dark.

D. simulans stock 1339.1a (table 2), as stated above, proved to be a refractory stock for the experiments. Out of 91 females, only three individuals were fecundated. Nevertheless, this stock maintains itself well in the laboratory.

Only one stock of *D. takahashii* was available for use. This stock breeds well in the laboratory cultures. The first series of experiments with this stock was conducted in the usual manner by placing 10 females with 5 males in an isolation vial after having aged the specimens for four days. Under varying lengths of exposure (table 3), none of the females exposed under conditions of total darkness was inseminated and even under lighted conditions the percentage of inseminations was low. In the seven-eight and ten day exposures, there was considerable mortality of the females, but none of the vials showed larvae and there is every reason to believe that those females which died before dissection were not inseminated. Since each species shows an optimum age for mating receptivity, it was considered possible that the four day old specimens had passed their peak of receptivity. One day old specimens, placed together under lighted conditions for one day exposure, gave 3.92 per cent fecundation, or 1.34 per cent lower than four day specimens when exposed to males for a period of equal length. Freshly emerged specimens placed together for six days, both in light and total darkness, showed clearly that this species could mate in the dark. The χ^2 (16.08) is significant, indicating perhaps some interference by darkness upon the mating process. Small mass matings of four day old individuals also show that matings are accomplished in the darkness, but indicate that by the fifth day after emergence this species is past its peak in receptivity. Mass matings, however, achieve a much higher percentage of insemination than do the 10:5 experiments for the same period of exposure to males. The present experiments are inadequate to elucidate the factor or factors that are responsible for these differences.

Small mass matings, consisting of freshly emerged individuals when exposed for six and seven days, gave relatively high percentages of insemination with very low and insignificant χ^2s. Five day exposure of 33 females under conditions of darkness resulted in no fecundations while 39 females under lighted con-

ditions showed 66.67 per cent insemination. It should be emphasized that in all cases the various experiments were done simultaneously in pairs, with specimens derived from the same stock cultures. Thus, as nearly as possible, all variables except light and darkness were eliminated.

Clearly *D. takashashii* can mate successfully in the dark. The present experiments show that the absence of light interferes with mating and that the intensity of the effect is determined by other factors. At present we cannot isolate these factors that act in connection with the absence of light and thus prevent or reduce inseminations. Specimens aged for four days and then placed in total darkness in the ratio of 10 females to 5 males do not mate even when kept together for ten days. If, however, small mass matings are utilized, then some four day old specimens will succeed in mating. Perhaps the density of the individuals, the greater number of males, or perhaps the effect of more individuals upon the food, or even other factors may be involved in causing such results. In comparison, individuals that are placed together immediately after emergence from the pupae but utilizing the 10:5 ratio mate more frequently than do individuals that have been aged for four days before being placed together. This is apparently due to the peak of mating receptivity in this stock being reached sometime during the first five days of adult life.

Discussion

Of the seven species tested only one, *D. auraria,* was completely inhibited from mating by total darkness. This is the second species of *Drosophila* now known that demands light in order to achieve copulation. Although it is possible that other species will be found that do not mate in the absence of light, certainly casual observations as well as specific but indirect evidence indicate that few "light dependent" species will be discovered. Both "light dependent" species now known, *D. subobscura* and *D. auraria,* belong to the sub-genus *Sophophora,* but belong to different species groups. The respective mating behaviors of the two species are quite different. Clearly the inability to mate in the dark has evolved independently in the two species.

D. rufa and *D. montium,* as shown above, have mating behaviors very similar to that of *D. auraria* but *D. montium* is not affected by darkness while the mating of *D. rufa* is somewhat curtailed by the absence of light. This seems to indicate that in the evolution of a closely related group of species with visibly similar mating behaviors, evolution has so proceeded that parallel types of stimuli are of different value to the various species. In the case of *D. subobscura,* Wallace and Dobzhansky (1946) clearly showed that males when observed in red light failed to copulate because they did not court. Hsu, working at Hangchow, China, showed that when using neutral red as a light filter *D. auraria* copulated at a greatly reduced rate. The present study gives no information as to why this species will not breed in the dark.

Pavan, Dobzhansky and Burla (1950), in their study of neotropical *Drosophila,* state that "apparently no species of *Drosophila* is active during the night," i.e., the flies do not move about during the night seeking feeding and ovipositing sites. Even though *Drosophila* may not be active in this sense at night, it does not necessarily follow that certain of their activities cannot occur in dark or semi-dark situations. The explanation of this apparent paradox is due to a number of interacting factors in the biology of the insects:

1. Drosophilid adults are attracted to various substances such as decaying fruits, rotting vegetables, tree sap, fungi, and in a few cases corollas of flowers (see Sturtevant, 1921; Patterson, 1943; and Carson and Stalker, 1950). In nature these substances occur typically as discrete units and the adult flies collect upon or in them to feed, mate and lay their eggs

since the larvae feed upon these substances and the accompanying microorganisms, especially the yeasts that are found within many of these materials.

2. Due to the interactions of light, temperature, and humidity the flies visit their feeding, mating, and breeding grounds only at specific limited times. In the temperate areas, as shown by Dobzhansky and Epling (1944) and as is well known to every collector of *Drosophila*, flies show a definite diurnal rhythm, coming to foods just before and for a couple of hours after sunrise and again just before sunset and until darkness. There is considerable specific variation, however, some species coming earlier or later than others and some staying for very short periods. If the food presents only an open surface, the flies leave it during the intervening times. If, however, the food presents enclosed cavities, e.g., holes in fruit due to the activity of birds or other agents, or if the food is enclosed in a cavity, e.g., bait containers, refuse cans, and perhaps cavities in trees in which fungi or fermenting substances have collected, then the flies will remain on or near the food during the day. Only if the temperature exceeds their range of tolerance will the flies leave such enclosed places since apparently the humidity and light intensity are such that the flies are not otherwise stimulated to leave. Pavan, Dobzhansky and Burla (l.c.) show that in southern and central Brazil "on clear, warm and dry days" the flies are active in the mornings and before sunset but that "in humid rain forests with equable temperatures, or on rainy days, the flies are active throughout the day."

In cavities or containers such as described above, the light intensity is greatly reduced and often completely absent. Yet it is in such places that the flies feed, mate, and oviposit. Obviously it is of adaptive value for such species as regularly utilize such places to be able to mate in the absence of light.

As to the adaptive value, if any, gained by the two species that mate only in the light, we have no available data. *D. subobscura* is found in England and Europe, while *D. auraria* is found in China, Japan and Formosa where it is an exceedingly common species, even more common than *D. melanogaster*. One of us (T. C. H.) has collected it from many localities in China and noted that it occurs mainly around marshy, damp habitats and beside bodies of water.

Summary

Study of seven species of the melanogaster species group of the subgenus *Sophophora* shows that *D. auraria* cannot mate in total darkness. Its close relative *D. rufa* can mate in the dark, but is inhibited under such conditions. A third closely related species, *D. montium*, copulates freely in darkness.

D. ananassae, *D. takahashii*, and *D. melanogaster* mate freely in total darkness but *D. simulans*, a close relative of *D. melanogaster*, is greatly inhibited by darkness.

Only one other species besides *D. auraria* (i.e., *D. subobscura*) is known to be unable to mate in the absence of light. Phylogenetic evidence shows that these two species have evolved this feature independently.

No evidence is available to show what, if any, adaptive value is attached to the type of mating behavior shown by *D. auraria* and *D. subobscura*, although evidence from a variety of sources indicates that there is an adaptive value to *Drosophila* being able to mate in the absence of light.

Selected Bibliography

Carson, Hampton L., and Harrison D. Stalker. 1950. Natural breeding sites for *Drosophila robusta*. Genetics, **35** (1): 100.

Dobzhansky, Th., and Carl Epling. 1944. Taxonomy, geographic distribution, and ecology of *Drosophila pseudoobscura* and its relatives. Carnegie Inst. Washington, Publ. **554**: 1–46.

Hsu, T. C. 1949. The external genital apparatus of male Drosophilidae in relation to systematics. Univ. of Tex. Publ. **4920**: 80–142.

PATTERSON, J. T. 1943. Studies in the genetics of Drosophila. III. The Drosophilidae of the southwest. Univ. of Tex. Publ. **4313**: 1–216.

PAVAN, C., TH. DOBZHANSKY, AND H. BURLA. 1950. Diurnal behavior of some neotropical species of Drosophila. Ecology, **31** (1): 36–43.

PAYNE, FERNANDUS. 1910. Forty-nine generations in the dark. Biol. Bull., **18**: 188–190.

PHILIP, U., J. M. RENDEL, H. SPURWAY, AND J. B. S. HALDANE. 1944. Genetics and karyology of *Drosophila subobscura*. Nature, **154**: 260–262.

RENDEL, J. M. 1945. Genetics and cytology of *Drosophila subobscura*. II. Normal and selective matings in *Drosophila subobscura*. J. Genetics, **26**: 287–302.

STURTEVANT, A. H. 1921. The North American species of Drosophila. Carnegie Inst. Washington, Publ. **300**: 1–150.

WALLACE, BRUCE, AND TH. DOBZHANSKY. 1946. Experiments on sexual isolation in Drosophila. VIII. Influence of light on the mating behavior of *Drosophila subobscura*, *Drosophila persimilis*, and *Drosophila pseudoobscura*. Proc. Natl. Acad. Sci., **32** (8): 226–234.

WHEELER, MARSHALL R. 1949. Taxonomic studies on the Drosophilidae. Univ. of Tex. Publ. **4920**: 157–195.

MEASUREMENT OF SEXUAL ISOLATION AND SELECTIVE MATING

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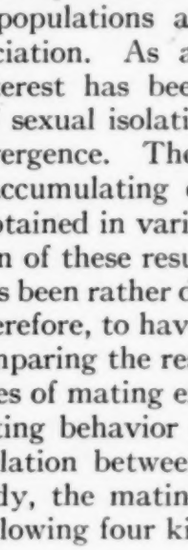
INTRODUCTION

The recent biological definitions of species by Dobzhansky (1941), Mayr (1942), and others have stressed the attainment of reproductive isolation between two populations as the critical step in speciation. As a result, considerable interest has been aroused in the study of sexual isolation at various levels of divergence. The data, which have been accumulating quite rapidly, have been obtained in various ways, but interpretation of these results on a common basis has been rather difficult. It is desirable, therefore, to have some simple means of comparing the results from the different types of mating experiments.

If the mating behavior or the degree of sexual isolation between two groups is under study, the mating possibilities are of the following four kinds.



With the male type designated first, these possibilities may conveniently be written AA (1) and BB (4) (homogamic matings) and AB (2) and BA (3) (heterogamic matings). Experimentally (and under natural conditions), various mating situations may exist. Each of the four matings may be studied separately, a method generally referred to as "pair matings." In a second experimental situation, males of one type may be placed with females of both types. This kind of experiment, the one most commonly used, may be called "male multiple choice." Similarly, females of one type may be placed with both types of males, a method which may be called "female multiple choice." Finally, both types of males and both types of females may be put together, probably most nearly approaching natural conditions. This situation perhaps may best be called simply "multiple choice."

Various indices have been proposed for the measurement of sexual isolation (Stalker, 1942; Levene and Dobzhansky, 1944; Bateman, 1949; Levene, 1949), but all have been designed for use with "male multiple choice" experiments. The significance of the simple indices with regard to sexual isolation has often been obscured by "one-sided mating preferences" (Dobzhansky, 1944) or differences in "relative mating propensity" (Bateman, 1949). This difficulty has been somewhat overcome by the use of a joint isolation index (Bateman, 1949) or a coefficient of joint isolation (Levene, 1949). However, the averaging of the two simple indices to give a single joint index in turn obscures the effects of selective mating. Bateman has taken this fact into account by deriving an index of female mating propensity.

Any analysis of data from mating experiments, therefore, should separate the effects of sexual isolation from those of various sorts of selective mating. Two measurements are desirable, one of the degree of sexual isolation, the other of the strength of selection for one type or the other.

SEXUAL ISOLATION

The measurement of sexual isolation involves making an estimate of the genetic isolation of one group from the other. This estimate should take into

account the possibility of gene flow in either or both directions since unidirectional gene flow will prevent the attainment of any degree of genetic diversity between the two groups about as effectively as gene flow in both directions.

A second requirement for an isolation index is that the effects of selective mating be minimized so that only those factors leading to true sexual isolation will affect the size of the figure obtained. Furthermore, applicability of the index to data from the various types of experiments will greatly enhance its usefulness.

A convenient measure of the degree of sexual isolation between two groups may be obtained from the following equation:

$$(1) \qquad I = \frac{AB + BA}{AA + BB}.$$

This term may be called the *isolation estimate.* It is designed for use when equal numbers of the types compared are present (for example, equal numbers of each female type in male choice experiments). This situation has been used in practically all experiments. However, since Levene and Dobzhansky (1945) have shown that the ratio of the probability of a heterogamic mating to the probability of a homogamic mating is a fixed constant relatively independent of the proportions of individuals, the value of I obtained in this way would appear to be generally descriptive of the sexual isolation between the groups. It will be seen that I is the ratio of heterogamic matings to homogamic matings. If there is no interbreeding between the groups so that they are completely sexually isolated, the value of I will be zero. On the other hand, if there is no sexual isolation, I will be approximately one. There would be no upper limit to I if both types of heterogamic matings occurred more often than the homogamic matings. However, this situation, which is quite improbable, has not yet been found experimentally so that in practice the value of I will vary from zero for complete isolation to one for random mating. The statistical significance of values intermediate between zero and one can be readily determined by the usual chi-square test.

The effects of different "mating propensities" by the female types or by the male types are, in effect, cancelled out because both types of females and both types of males appear in both numerator and denominator.

Levene (1949) has pointed out that different durations for reciprocal mating experiments may influence the joint isolation index. His coefficient of joint isolation is designed to minimize this effect. In the calculation of I also, the effect of different exposure times in reciprocal male or female multiple choice experiments is somewhat offset. The male multiple choice experiments may be used to demonstrate this fact. The basis of I in that case is the difference between the female types in frequency of mating. Because the sum of the times of exposure for both female types is equal, and the sum of these periods in the numerator equals that in the denominator, it can be seen that I is relatively insensitive to differences in the duration of the reciprocal experiments. With "multiple choice" tests the time, of course, is the same for all types. The calculation of I from data of pair matings is most susceptible to spurious fluctuations due to different exposure times.

Selective Mating

For the evaluation of mating data, some measure of the degree of selective mating is also desirable. Bateman (1949), recognizing this need, derived a *mating propensity index* for the special case of male multiple choice experiments. This index measures the relative mating propensities of the females. A similar index for the males would be useful.

Male multiple choice experiments furnish information only about the relative mating frequencies of the females, and female multiple choice experiments only

about those of the males. Indices of both types can be derived from multiple choice tests and from pair matings, but the time factor must be controlled with the latter for significant results.

The relative frequency of mating by the two female types may be determined from the equation:

$$(2) \qquad M_f = \frac{\text{AA} + \text{BA}}{\text{BB} + \text{AB}}.$$

This relationship may be called the *female mating ratio.* Similarly, the *male mating ratio* is expressed as

$$(3) \qquad M_m = \frac{\text{AA} + \text{AB}}{\text{BB} + \text{BA}}.$$

These values are also designed to be used with the most common experimental situation in which the numbers of the types being tested are equal. If types A and B differ in a single allele which is responsible for these mating differences, and the value of I indicates that there is no sexual isolation, the mating ratios can be used to estimate the changes in gene frequency in a population due to selective mating. In this case it is assumed that there is a fixed probability that an individual of a given type will mate. The probability of a given mating between a male and a female is then the product of these separate probabilities. This assumption may not be completely valid, but it has been extremely useful in predicting trends due to selective mating in populations.

For the case of an autosomal recessive, with the heterozygote behaving like the homozygous dominant, the following equations may be derived.

Using symbols drawn from Hogben (1946), let

D_m = dominant males
D_f = dominant females
R_m = recessive males
R_f = recessive females
H_m = heterozygous males
H_f = heterozygous females

$$D_m + R_m + H_m = 1.0$$
$$D_f + R_f + H_f = 1.0$$

Then,

$$(4) \quad R_{n+1} = \frac{(\frac{1}{2}H_{m_n} + M_m R_{m_n}) \times (\frac{1}{2}H_{f_n} + M_f R_{f_n})}{(1 - R_{f_n} + M_f R_{f_n}) \times (1 - R_{m_n} + M_m R_{m_n})},$$

$$(5) \quad D_{n+1} = \frac{(D_{m_n} + \frac{1}{2}H_{m_n})(D_{f_n} + \frac{1}{2}H_{f_n})}{(1 - R_{f_n} + M_f R_{f_n}) \times (1 - R_{m_n} + M_m R_{m_n})},$$

$$(6) \quad H_{n+1} = 1 - R_{n+1} - D_{n+1}.$$

The value for R_{n+1} (similarly for D_{n+1} and H_{n+1}) can be substituted for both R_m and R_f since the gene is autosomal.

The equations are somewhat simpler if there is deviation from random mating in only one sex. If nonrandom mating is found only in the males, for example, the equations become

$$(7) \quad R_{n+1} = \frac{(\frac{1}{2}H_{m_n} + M_m R_{m_n})(\frac{1}{2}H_{f_n} + R_{f_n})}{1 - R_{m_n} + M_m R_{m_n}},$$

and so forth.

For a sex-linked recessive, the proportions of the various male and female genotypes in each generation can be obtained as follows:
Let

RR = recessive females
RY = recessive males
RD = heterozygous females
DD = dominant females
DY = dominant males

$$RR + RD + DD = 1.0$$
$$RY + RD = 1.0$$

Then,

$$(8) \quad RY_{n+1} = \frac{\frac{1}{2}RD_n + M_f RR_n}{1 - RR_n + M_f RR_n},$$

$$(9) \quad DY_{n+1} = 1 - RY_{n+1},$$

$$(10) \quad RR_{n+1} = \frac{M_m RY_n}{DY_n + M_m RY_n} \cdot RY_{n+1},$$

$$(11) \quad DD_{n+1} = \frac{DY_n}{DY_n + M_m RY_n} \cdot DY_{n+1},$$

$$(12) \quad RD_{n+1} = 1 - RR_{n+1} - DD_{n+1}.$$

If only the males are selectively eliminated, a situation which has been found to be quite common experi-

mentally, the equations are as follows: Let

p_f = frequency of dominant gene in the females,

q_f = frequency of recessive gene in the females,

$p_m = DY$ = frequency of dominant gene in the males,

$q_m = RY$ = frequency of recessive gene in the males.

Then,

$$(13)\quad RY_{n+1} = q_{f_n} = q_{m_{n+1}},$$

$$(14)\quad DY_{n+1} = p_{f_n} = p_{m_{n+1}},$$

$$(15)\quad RR_{n+1} = \frac{M_m q_{m_n} q_{f_n}}{p_{m_n} + M_m q_{m_n}},$$

$$(16)\quad DD_{n+1} = \frac{p_{m_n} p_{f_n}}{p_{m_n} + M_m q_{m_n}},$$

$$(17)\quad RD_{n+1} = 1 - RR_{n+1} - DD_{n+1}$$

$$(18)\quad q_{f_{n+1}} = RR_{n+1} + \tfrac{1}{2} RD_{n+1},$$

$$(19)\quad p_{f_{n+1}} = 1 - q_{f_{n+1}}.$$

From equation (13) it can be seen that the frequency of recessive males in generation $n + 1$ equals the frequency of the recessive gene in the females of the previous generation, n.

Calculation of I, M_f and M_m

Table 1 has been drawn up to illustrate the calculation of the three ratios from all four types of mating experiments. The more common results showing sexual isolation and selective mating are demonstrated.

The multiple choice data of Reed and Reed (1950) with white-eyed and wild type *Drosophila melanogaster* show that the cause of the deviations from random mating is the relative inability of the white-eyed males to obtain mates as compared with the wild type.

Mayr and Dobzhansky's work (1945) with *Drosophila pseudoobscura* and *Drosophila persimilis* shows a high degree of sexual isolation. There is a tendency for *pseudoobscura* females to mate more

Table 1

Type of experiment	Source	Female type	Male types		I	χ^2	M_f	χ^2	M_m	χ^2
Multiple choice	Reed and Reed (1950) Table 1	white eyed heterozygous	white eyed 90 64	wild type 100 105	0.84	2.68	1.12	1.23	0.75	7.24†
Male multiple choice	Mayr and Dobzhansky (1945) Table 2	*pseudoobscura* *persimilis*	*pseudoobscura* 72/82 8/83	*persimilis* 27/119 81/127	0.24	70.19†	1.16	1.07	—	—
	Dobzhansky and Streisinger (1944) Table 1	Chilpancingo Huichihuayan	Chilpancingo 69/72 23/70	Huichihuayan 41/47 15/48	0.78	2.22	2.87	34.37†	—	—
	Patterson, McDanald and Stone (1947) Table 4	*americana* *texana*	*americana* 70/115 36/115	*texana* 62/112 34/111	0.94	0.16	1.88	18.78†	—	—
	Tan (1946) Table 1	*y sn v co sh* *ast*	*y sn v co sh* 15/44 2/46	*ast* 26/39 23/38	0.71	1.92	1.66	4.08*	—	—
	Merrell (1949) Table 3	cut heterozygous	cut 15 24	wild type 11 22	0.95	0.06	0.57	5.56*	—	—
Female multiple choice	Merrell (1949) Table 2	cut heterozygous	cut 3 6	wild type 23 41	0.66	3.08	—	—	0.14	41.44†
Pair mating	‡Patterson, Stone and Griffen (1940) Table 1	*americana* *texana*	*americana* 74 28	*texana* 44 54	0.56	15.68†	1.44	6.48*	1.04	0.08

* Statistically significant.
† Statistically highly significant.
‡ Based on counts of at least 100 vials. χ^2 on that basis.

often than *persimilis* females, but it is not statistically significant in this case.

The results with the Chilpancingo and Huichihuayan strains of *Drosophila prosaltans* show that, although the groups are not sexually isolated, the Chilpancingo females mate much more frequently than Huichihuayan females. Tan's data with mutants of *Drosophila pseudoobscura* and the data on male choice for *cut* in *Drosophila melanogaster* are similar to the *prosaltans* results, one female type mating more often than the other. These similarities may or may not have similar causes. For Tan's data, Bateman's joint isolation index gives a value of 0.37, from which he concludes that "The overall effect of this one-sided action of *y* on mating behavior is the sexual isolation of *y*." Actually, only one type of mating is blocked, that between *y sn v co sh* males and *ast* females, but the exchange of genes is still possible via the reciprocal mating. Therefore, these groups should not be considered sexually isolated to any great extent, and a situation of this kind will never lead to complete sexual isolation.

The value of I for *cut* obtained from female multiple choice experiments differs from that obtained from male choice experiments, but neither is statistically significant. However, M_m shows the notable lack of mating success by the *cut* males, and M_f a lower mating frequency for *cut* females.

The data (1940) on pair matings between *Drosophila americana americana* and *Drosophila americana texana* (see Stone and Patterson, 1947) show that the *americana* females mate somewhat more often than the *texana* females. Furthermore, even though natural hybrids are still found, these subspecies are already to some extent sexually isolated. These results can be compared to the data from male multiple choice experiments of Patterson, McDanald, and Stone (1947). Again the *americana* females mate more frequently than *texana* females, but in this case, no sexual isolation is evident. This difference is not surprising since Streisinger (1948) demonstrated that Drosophila males show relatively little discrimination in mating when given a "choice" of two species of etherized females. Therefore, sexual isolation between closely related groups is evidently less apt to show up in experiments with male choice.

Summary

For the measurement of the sexual isolation between two populations, an *isolation estimate* is derived for use with data from many kinds of mating experiments. This *isolation estimate* is relatively insensitive both to the effects of selective mating and to the effects of different durations of exposure of females to males in reciprocal experiments, thus providing a consistent estimate of the degree of isolation.

A *female mating ratio* and a *male mating ratio* are derived to measure the relative frequency of mating by the different types of the same sex. These ratios are further used in equations for the calculation of the effect of selective mating on gene frequencies when the two types differ at one locus.

Typical examples are chosen from the various kinds of mating experiments to illustrate the calculation and interpretation of these three terms.

Acknowledgment

I wish to thank Dr. Howard Levene for his helpful criticisms of the manuscript.

Literature Cited

Bateman, A. J. 1949. Analysis of data on sexual isolation. Evolution, **3**: 174–177.

Dobzhansky, Th. 1941. Genetics and the origin of species, New York: Columbia University Press.

Dobzhansky, Th. 1944. Experiments on sexual isolation in *Drosophila*. III. Geographic strains of *Drosophila sturtevanti*. Proc. Nat. Acad. Sci., **30**: 335–339.

Dobzhansky, Th., and G. Streisinger. 1944. Experiments on sexual isolation in *Drosophila*. II. Geographic strains of *Drosophila prosaltans*. Proc. Nat. Acad. Sci., **30**, 340–345.

HOGBEN, L. 1946. An introduction to mathematical genetics. New York: W. W. Norton Co.

LEVENE, H. 1949. A new measure of sexual isolation. Evolution, **3**: 315–321.

LEVENE, H., AND TH. DOBZHANSKY. 1945. Experiments on sexual isolation in *Drosophila*. V. The effect of varying proportions of *Drosophila pseudoobscura* and *Drosophila persimilis* on the frequency of insemination in mixed populations. Proc. Nat. Acad. Sci., **31**: 274–281.

MAYR, E. 1942. Systematics and the origin of species. New York: Columbia University Press.

MAYR, E., AND TH. DOBZHANSKY. 1945. Experiments on sexual isolation in *Drosophila*. IV. Modification of the degree of isolation between *Drosophila pseudoobscura* and *Drosophila persimilis* and of sexual preferences in *Drosophila prosaltans*. Proc. Nat. Acad. Sci., **31**: 75–82.

MERRELL, D. J. 1949. Selective mating in *Drosophila melanogaster*. Genetics, **34**: 370 389.

PATTERSON, J. T., L. W. MCDANALD, AND W. S. STONE. 1947. Sexual isolation between members of the *virilis* group of species. Univ. Texas Publ., **4720**: 7–31.

PATTERSON, J. T., W. S. STONE, AND A. B. GRIFFEN. 1940. Evolution of the *virilis* group in *Drosophila*. Univ. Texas Publ., **4032**: 218–250.

REED, S. C., AND E. W. REED. 1950. Natural selection in laboratory populations of *Drosophila*. II. Competition between a white-eye gene and its wild type allele. Evolution, **4**: 34–42.

STALKER, H. D. 1942. Sexual isolation studies in the species complex *Drosophila virilis*. Genetics, **27**: 238–257.

STONE, W. S., AND J. T. PATTERSON. 1947. The species relationships in the *virilis* group. Univ. Texas Publ., **4720**: 157–160.

STREISINGER, G. 1948. Experiments on sexual isolation in *Drosophila*. IX. Behavior of males with etherized females. Evolution, **2**: 187–188.

TAN, C. C. 1946. Genetics of sexual isolation between *Drosophila pseudoobscura* and *Drosophila persimilis*. Genetics, **31**: 558–573.

STUDIES OF VARIATION IN THE WEED GENUS *PHYTOLACCA*. I. HYBRIDIZING SPECIES IN NORTHEASTERN COLOMBIA

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INTRODUCTION

Phytolacca is one of the many plant genera whose members have spread and multiplied with the human race, not because they were taken into cultivation and deliberately propagated, but because they were able to colonize places where man had scarred or destroyed the natural vegetative cover. The story of these weeds is an important problem both in evolution and in ethnobotany, but it is extremely difficult to trace in many genera because of their wide migrations and complex hybridization. *Phytolacca* offers peculiarly attractive material for an attack on this problem. Although characteristically weeds of artificial habitats, the members of this genus are remarkable among weeds by the simplicity of their taxonomy and their lack of aggressive migration beyond their native ranges.

The present paper considers variation in a zone of contact between two tropical American species. Succeeding papers will deal with variation patterns in temperate North America, where a single species is at present almost completely isolated.

The collections and field observations on which this paper is based were made in 1944 by the senior author while on the Colombian Cinchona Mission in the departments of Santander and Norte de Santander. The material was analyzed by the junior author under the supervision of Dr. Herbert Mason and Dr. G. Ledyard Stebbins at the University of California and Dr. Edgar Anderson at the Missouri Botanical Garden. Dr. Anderson's influence on the graphic presentation will be obvious to those acquainted with his work.

SPECIES INVOLVED

In his monograph on the *Phytolaccaceae*, H. Walter (1909) reported only one species of *Phytolacca* from Santander and Norte de Santander, although he reported six species from the whole of Colombia. The present collections include four species. Two of these, *P. octandra* L. and *P. icosandra* L., are represented by only a few specimens and will not be discussed here. The great bulk of the present collections are either clearly assignable to two species, *P. rivinoides* Kunth et Bouché and *P. rugosa* A. Br. et Bouché, or appear to be intermediate between the two.

Phytolacca rivinoides and *P. rugosa* are generally regarded as good and distinct species; no mention of intergrades or hybrids has been found in any published literature. Walter placed them in separate subgenera and gave the following character differences:

	P. rivinoides	*P. rugosa*
Number of stamens	10–20	8
Number of carpels	10–16	8
Carpels connate	Completely	Base only
Tepals (Perianth parts)	Caducous	Persistent
Inflorescence length	40 cm.	9 cm.
Pedicel length	10 mm.	5 mm.
Tepal length	2 mm.	3 mm.

An examination of the species in the field and herbarium confirms their strong divergence. *P. rugosa* usually has about eight carpels and stamens, occasionally as few as six or as many as nine. This slight variation is probably not due to genetic differences, since it is commonly observed among different flowers on the same plant. *P. rivinoides* typically has sixteen or more carpels and stamens, although there is fairly wide variation in number within a single inflorescence. The two species differ sharply in several other flower characters. *P. rivinoides* has completely connate carpels not easily counted at anthesis except by sectioning; its styles are slender, erect, and appressed (fig. 1). *P. rugosa* has carpels connate only at the

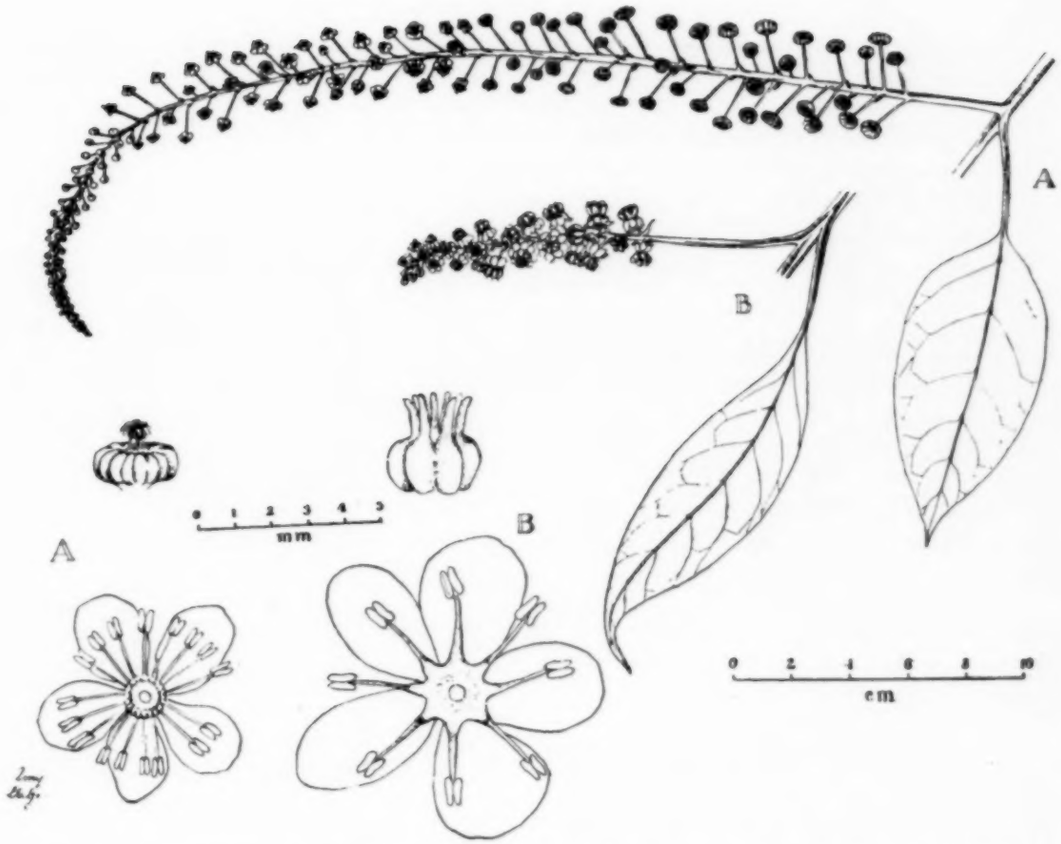

FIG. 1. Comparison of flower and inflorescence characters of *P. rivinoides* (A) and *P. rugosa* (B)

bases, with deep grooves between adjacent carpels; its styles are stout and strongly recurved. *P. rivinoides* has the stamens attached to the disc in one or two rather indefinite whorls; thickening of the disc forms a circular ridge within the point of staminal insertion (fig. 1). *P. rugosa* has a definite inner whorl of stamens; occasional extra stamens clearly belong to a distinct outer whorl; the thickening is concentrated on the bases of the filaments.

In addition to these almost microscopic differences in flower structure, the species are strikingly unlike in more conspicuous characters. *P. rivinoides* has light purple flowers, with small thin tepals which drop off before the fruit matures; in pressed specimens the tepals are pale yellow. *P. rugosa* has deep purplish-red flowers; the tepals are long, thick, and persistent; they retain a reddish tinge in herbarium specimens. The mature inflorescence of *P. rivinoides* is a long loose raceme, or not uncommonly a panicle, usually at least twice as long as the adjacent leaf; the peduncle forms only a small fraction of the total inflorescence length (fig. 1). *P. rugosa* has a small compact raceme, commonly shorter than the adjacent leaf; the penduncle is ordinarily as long as the rest of the inflorescence. *P. rivinoides* has very long slender pedicels, often more than 15 mm. long; *P. rugosa* has short stout pedicels, rarely more than 5 mm. long.

The two species tend to differ in other quantitative characters, but these are dependent on stage of maturity and environmental effects to such a degree that they are difficult to analyze.

THE COLLECTIONS AND THEIR ANALYSIS

Collecting could not be done very systematically, since duties on the cinchona mission constituted a full-time job, but the habitats of *Phytolacca* are such as to

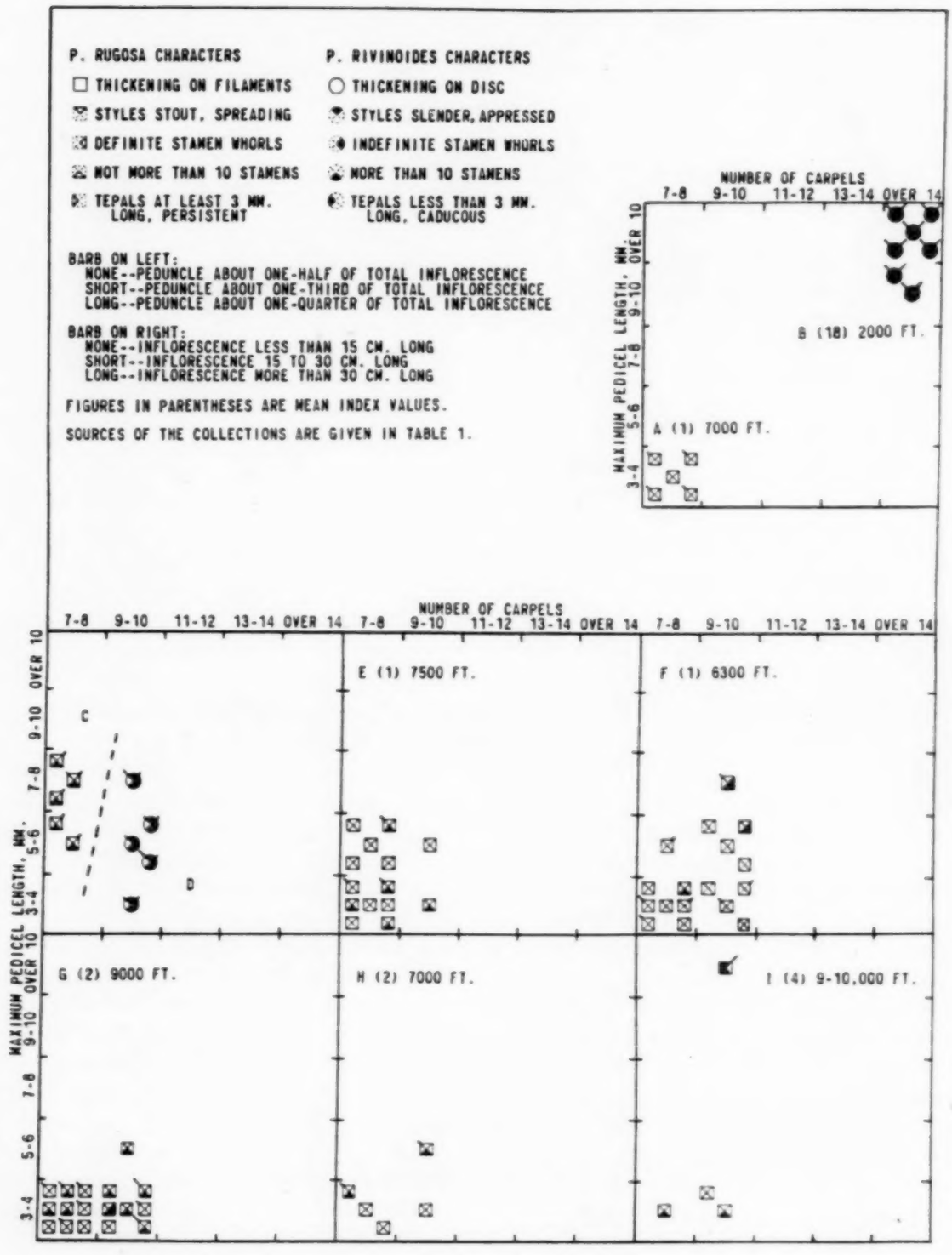
P. RUGOSA CHARACTERS
THICKENING ON FILAMENTS
STYLES STOUT, SPREADING
DEFINITE STAMEN WHORLS
NOT MORE THAN 10 STAMENS
TEPALS AT LEAST 3 MM. LONG, PERSISTENT
P. RIVINOIDES CHARACTERS
THICKENING ON DISC
STYLES SLENDER, APPRESSED
INDEFINITE STAMEN WHORLS
MORE THAN 10 STAMENS
TEPALS LESS THAN 3 MM. LONG, CADUCOUS
BARB ON LEFT:
NONE--PEDUNCLE ABOUT ONE-HALF OF TOTAL INFLORESCENCE
SHORT--PEDUNCLE ABOUT ONE-THIRD OF TOTAL INFLORESCENCE
LONG--PEDUNCLE ABOUT ONE-QUARTER OF TOTAL INFLORESCENCE
BARB ON RIGHT:
NONE--INFLORESCENCE LESS THAN 15 CM. LONG
SHORT--INFLORESCENCE 15 TO 30 CM. LONG
LONG--INFLORESCENCE MORE THAN 30 CM. LONG
FIGURES IN PARENTHESES ARE MEAN INDEX VALUES.
SOURCES OF THE COLLECTIONS ARE GIVEN IN TABLE 1.
NUMBER OF CARPELS
7-8 9-10 11-12 13-14 OVER 14
MAXIMUM PEDICEL LENGTH, MM.
3-4 5-6 7-8 9-10 OVER 10
A (1) 7000 FT.
B (18) 2000 FT.
C
D
E (1) 7500 FT.
F (1) 6300 FT.
G (2) 9000 FT.
H (2) 7000 FT.
I (4) 9-10,000 FT.

FIG. 2a.

Character analysis of mass collections of *P. rivinoides–P. rugosa* series from Santander and Norte de Santander.

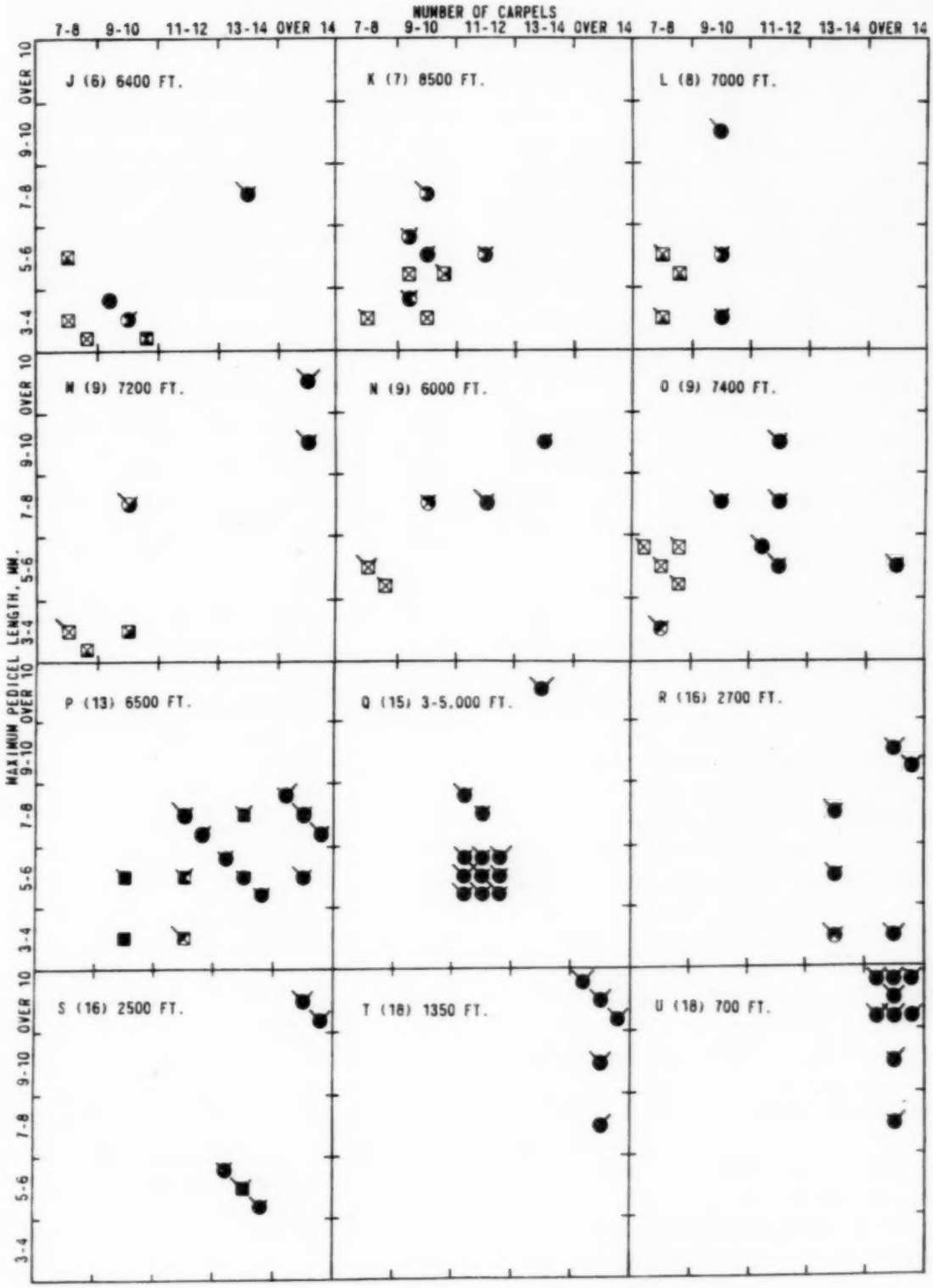

FIG. 2b.

Character analysis of mass collections of *P. rivinoides–P. rugosa* series from Santander and Norte de Santander.

make possible the snatching of a fragment from each plant while walking a trail or climbing through a cornfield. A short length of stem with leaf and raceme attached was taken from each plant. A total of about 200 plants from about 30 localities was available for analysis.

Plants were scored individually for a selected group of characters and the data plotted in the forms of ideographs, as explained in figure 2. Stamen, disc, style, and tepal characters were scored in only two ways—similar to *P. rugosa* or similar to *P. rivinoides*—since there were few apparent intermediates. Inflorescence length and peduncle length exhibited many intermediate gradations; however, these characters are so subject to non-genetic variability that only two extremes and one intermediate category are represented in the symbols. Pedicel length and carpel number show a series of gradations which appear to be quite constant on individual plants; these gradations are represented by the horizontal and vertical positions of the symbols on the graphs.

Aggregate index values were assigned to each plant and from these means were computed for each collection. In computing the index values, two points were assigned for each character like *P. rivinoides*, one point for intermediate characters, and zero for characters like *P. rugosa*. Index values have a possible range from 18 in an ideal *P. rivinoides* type to 0 in an ideal *P. rugosa* type.

The first graph shows two of the most extreme index collections; collection A approaches pure *P. rugosa* and collection B approaches pure *P. rivinoides*. Collections C and D represent multiple specimens from two individual plants which were scored in the mistaken belief that they were mass collections; they are included here as an indication of the nature and amount of variation in diagnostic characters to be found within one plant. Collections E to U show all the other larger collections from this region, arranged in order of their mean index values. Their sources are given in table 1. Collections of less than four plants are not graphed separately, but are included in the tabulations of the total collection (figs. 3, 4).

The striking variability of most of the intermediate populations contrasts sharply with the uniformity of the extreme populations. Some of the intermediate collec-

TABLE 1. *Collections of* P. rugosa—P. rivinoides *series from Santander (S) and Norte de Santander (NS)*

Field number	Graph	Locality
Collections represented on graphs:		
26004	A	Near Quebrada Cueva on Rio Cucutilla (NS)
25595	B	Near Placitas, El Carmen Region (S)
25828	C	East of Florian (S)
25801	D	4 km. E of La Belleza (S)
25774	E	La Belleza (S)
25988	F	23 km. N of Toledo on Rio Culaga (NS)
25486	G	17 to 18 km. N of Vélez (S)
25100	H	10 km. NW of Vélez on road to Bolívar (S)
25128	I	5 to 10 km. W of Bolívar (S)
25181	J	Between Canta Ranas and Agua Blanca, 5 km. N of La Paz (S)
25957	K	Echeverria estate, E of Toledo (NS)
25802	L	4 km. E of La Belleza (S)
25147	M	La Sabaneta, 5 km. SW of La Paz (S)
26035–6	N	Above Villa Marina, N of Pamplona (NS)
25982	O	Aguacaliente, on Rio Culaga, 20 km. N of Toledo (NS)
25952	P	Near Bata, S of Toledo (NS)
25776	Q	Near Chiquinquirá, 8 to 14 km. SW of La Belleza (S)
25234	R	Several km. area, La Venezia, near Jordán (S)
25777	S	Near Florián (S)
25346	T	La Quitiana, near Cimitarra (S)
25375	U	5 km. W of Puerto Aquileo (S)
Small collections not graphed separately:		
25077		El Retiro, 18 km. N of Vélez (S)
25140		El Espejo, 4 km. SW of La Paz (S)
25199		5 km. W of La Paz on road to Recreo (S)
25218		Between Recreo and Mira Buenos, W of La Paz (S)
25240		La Soledad, 20 km. NE of Jordán (S)
25550		W of Chima (S)
25583		W of Chima (S)
25590		Near Rio Oponcito, 25 km. W of San Vincente (S)

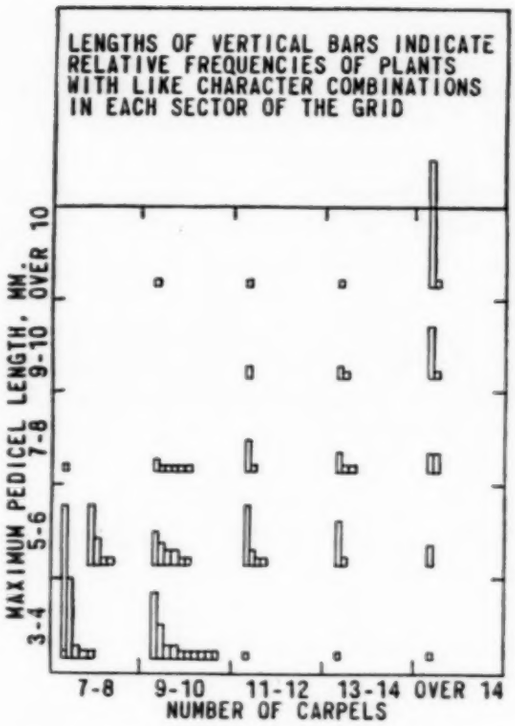

FIG. 3. Number of plants with like character combinations in combined collections of *P. rivinoides–P. rugosa* series.

tions are apparently derived from hybrid swarms with a wide diversity of character recombinations; others apparently represent the result of repeated backcrossing to one or the other parental type, so that the only evidence of introgression is in the variability of a few characters. The uniformity of the extremes and variability of the intermediates is even more evident when all the collections are combined, as in figure 3, in which the total number of individuals is plotted against the number of character combinations. These results definitely indicate character recombinations produced by mixing, rather than an ancient clinal gradient.

Although a great variety of recombinations occur, the character combinations of the parental types are seldom completely shattered. The parental complexes of tepal, style, and disc morphology are most coherent and only occasionally recombine in the hybrids. Other characters, such as lengths of inflorescence, length of pedicels, and number of carpels and stamens, are apparently easily modified by introgression.

FIELD OBSERVATION OF HABITAT

Pure colonies of *P. rugosa* were found only in the *tierra templada,* from approximately 6,000 to 9,000 feet altitude, while *P. rivinoides* in its typical form was found only in the *tierra caliente,* below 5,000 feet altitude. There is a rough but definite correlation between altitude and the index values of the colonies (fig. 4).

In this region, as elsewhere, *Phytolacca* is seldom seen in undisturbed habitats. Both species are locally abundant along the few highways and the more extensive mule trails. They are common in cultivated fields, particularly in temporary mountain clearings. To make these clearings the trees are cut on a patch of a few acres, allowed to dry, and burned. After a few years of cultivation the soil is gone, the land is abandoned, and a new clearing is made. There is no mechanical cultivation and apparently no hand weeding, so weeds, including *Phytolacca,* become established

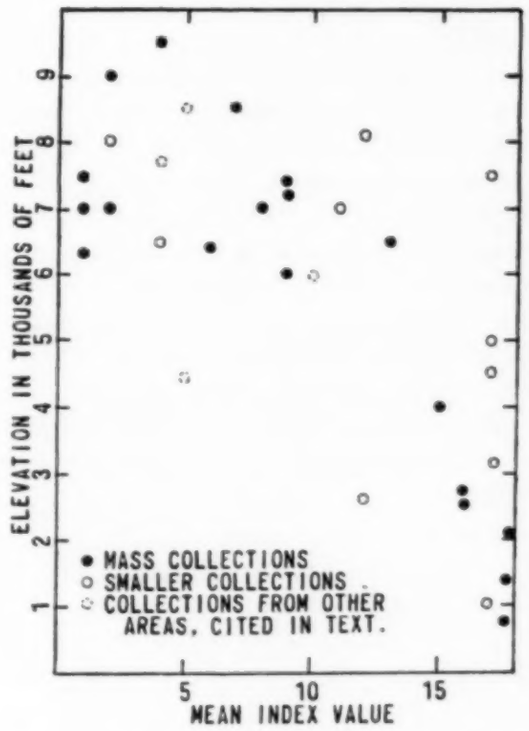

FIG. 4. Relation of index value to elevation in *P. rivinoides–P. rugosa* series.

while the clearings are still planted to crops. *Phytolacca* appears to be able to survive for at least a few years in the abandoned clearings after artificial disturbance of the habitat has ceased.

The three months spent by the senior author in the general area of the Carare Road in the southern part of Santander made possible some fairly detailed observations on the local occurrence of *Phytolacca*. The Carare Road runs northwestward from the railway terminal at Barbosa, to Vélez, Jordán, Landázuri, Puerto Aquileo, and thence westward to Puerto Berrío on the Magdalena River. From Vélez, at about 7,100 feet altitude, the road climbs to about 9,000 feet. Then (contrary to the available maps) it follows the narrow and steep-sided ridge between the headwaters of the Rio Horta and the Rio Opón to Jordán, apparently a recent settlement, at 2,600 feet. From Jordán the descent is rapid, through Landázuri at 2,000 feet, to Cimitarra at 1,000 feet. Cimitarra is scarcely a town, but is the location of a rest-camp for the laborers whose duty it is to clear the frequent landslides and keep the Carare Road open as much of the time as possible. Here the mountains abruptly stop and the road comes out on the flat plains of the Magdalena River.

From Vélez, for 20 km. northward as the road winds, *Phytolacca* is a common roadside plant. As a passenger on an onion truck, with nothing better to do, the author once counted 49 plants (or dense clumps of plants) in the 20 km. All had short dense racemes of dark-colored flowers, and appeared to be *P. rugosa*. Collection G is from the Carare Road near its highest point, and collection H is from the branch road to Bolívar. *P. rugosa* disappears 20 km. north of Vélez; then for 30 km. to Jordán, there is no *Phytolacca* along the road. At Jordán appear plants close to *P. rivinoides* (collection R), and the plants become purer as one follows the road to its crossing of the Carare River in the tropical zone near Puerto Aquileo (collections T, U).

The cline from pure *P. rivinoides* at 700 feet to the intermediate colony at 2,600 feet (collection R) is not due to present contact with *P. rugosa*, at least not along the Carare Road, where there is a gap of 30 km. between the two species. However, the Carare Road is of fairly recent construction. The slight infusion of *P. rugosa* in the *P. rivinoides* at Jordán must be a relic of spreading and mingling of *Phytolacca* in fields or along the 300 year old network of mule trails.

About La Paz, northeast of Vélez, mass collections are somewhat heterogeneous (collections J, M). La Paz is reached only by a mule trail; about the headwaters of the Rio Opón are many small towns connected only by narrow trails often worn deep into the bedrock. Settlement in this area is old, and *Phytolacca* may have been mixing for centuries.

From Florián, at the end of a road going west from Barbosa, the terrain falls off abruptly to the westward into the low subtropical country bordering the Rio Minero. There is also a sharp gradation from almost pure *P. rugosa* (collection E) at La Belleza to nearly pure *P. rivinoides* (collections Q, S) on a ridge at 2,500 feet, still considerably east of the low ground along the Rio Minero.

In Norte de Santander fewer collections were obtained, but here again both fairly pure and quite mixed colonies were found. For example, a colony of typical *P. rugosa* (collection F) was found in a clearing at Aguacaliente, north of Toledo; only 3 km. away in the same valley a colony was found which included a variety of forms ranging from typical *P. rugosa* to plants close to *P. rivinoides* (collection O).

Similar Hybrids in Other Areas

Mixing between these two species may occur over a wide area; they range together, though at different altitude levels, from Colombia and Venezuela up through Central America into southern Mexico. The available herbarium material is not

adequate to determine the extent of hybridization, but it is certainly not confined to the two Colombian departments studied. A mass collection from the Colombian Comisaría of Caquetá (H. L. Mason 13928) is close to *P. rugosa,* but shows unmistakable traces of *P. rivinoides.* Specimens from the province of Chiriquí in Panama (Bro. Maurice 742; M. E. Davidson 153) and the state of Chiapas in Mexico (E. Matuda 4,325) also are intermediate between the two species.

Conclusions

Field observation and mass collection analysis indicate that active mixing is taking place along the eastern Cordillera of Colombia between two native species of *Phytolacca.* Preliminary examination of herbarium material suggests similar hybridization elsewhere within the joint ranges of these species. The process is remarkable because of the extreme morphological and ecological divergence of the species involved. The two are as dissimilar in morphology as almost any of the dozen or so American species of the genus. One, in its purest phases, inhabits the broad plains along the Rio Magdalena at elevations of 300 to 1,000 feet; this is a tropical land of bananas and coconuts. The other is most abundant and most pure in the cooler parts of the temperate highlands at elevations of 7,000 to 9,000 feet, higher than the groves of mountain grown coffee and at the altitude where *Cinchona officinalis* grows.

It seems probable that in pre-human times the two were ecologically and spatially separated into clearcut lowland and highland species. With the coming of man and his disruption of the natural vegetation, they undoubtedly became much more abundant in their native altitude zones and migrated as occasional weeds into disturbed habitats at middle altitudes. Habitat disturbance may have functioned both by permitting spread of the original types beyond the zone of their highest adaptation and by favoring survival of hybrid recombinations wherever the two species came into contact.

Contact and the resulting mixing may have begun in ancient times with Indian agricultural activity or they may have followed changes in land use since the Conquest. In any case, there is no reason to believe that hybridization waited until modern times. Vélez was settled by the Spanish before 1600 and the present system of agriculture has been extant for centuries. The fact that the two species are still quite distinct except at middle altitudes may simply reflect the presence of climatic barriers. Climatic selection, operating directly on adaptive characters and indirectly on other characters bound to adaptive characters by linkage, could be expected to provide a strong shield against severe introgression. Species adapted to such extremely dissimilar native habitats could, under such climatic selection, maintain a fair degree of integrity in spite of prolonged contact and cross-fertilization.

Literature Cited

Walter, Hans. 1909. Phytolaccaceae; Das Pflanzenreich, ed. A. Engler, IV. 83.

THE GENETICAL ARCHITECTURE OF HETEROSTYLY IN *PRIMULA SINENSIS*

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THE COMPLEXITY OF HETEROSTYLY

Flowering plants show a variety of mechanisms which encourage, or even ensure, the setting of seed by cross- rather than by self-pollination. Heterostyly is such an outbreeding device and it is widespread in its occurrence. Heterostyled species may be classified into two groups, the distylic and tristylic, the former being the commoner type. In a distylic species the plants are of two kinds, differing in the flowers that they bear. The one type, termed "thrum," is recognizable by its anthers being borne above the level of its stigmata, while the other, or "pin" type, has the position of stigmata and anthers reversed, the former now being above the latter (see fig. 2). This is the type of heterostyly which occurs in species of *Primula* and with which the present account is concerned. Species showing the second type of heterostyly, tristyly, have plants of three kinds, distinguished by the level of the stigma being below, between, or above the levels of the two sets of anthers which are borne in such flowers. The details of tristyly need not, however, detain us in our consideration of distylic *Primulas*.

Distyly in *Primula* species is a complex character. The morphological duality involving position of anthers and position of stigma, is obvious to mere inspection, but the differences of structure do not stop there. The two types of stigma differ also in the size of their papillae, the pin stigma having larger ones than the thrum, and the stamens differ in the size of the pollen grains they produce, thrum pollen being larger in mean size and also less variable than pin. Finally, in addition to these morphological differences there is, as Darwin (1877) showed, a physiological distinction. Pollen, whether from pin or thrum flowers, is more successful in giving rise to seed on a stigma of the opposite type of flower than on one of its own type. There is an incompatibility reaction of pin pollen on pin stigmata, or perhaps more correctly of pin pollen tubes in pin styles, and of thrum pollen on thrum stigmata. We can thus recognize six sub-characters in heterostyly (Lewis 1949):

(i) Position of stigma.
(ii) Position of anthers.
(iii) Size of stigmatic papillae.
(iv) Size of pollen grains.
(v) Incompatibility reaction of pollen tube.
(vi) Incompatibility reaction of style.

These differences are normally switched as a single unit by the single gene *S–s;* an *Ss* (and, as has been shown experimentally in *Primula sinensis,* an *SS*) plant having all the six characteristics associated with the thrum type, and an *ss* plant having all the six alternatives associated with the pin type. It would nevertheless appear that the six sub-characters are not a single developmental unit (as are, for example, the various effects of Grüneberg's (1938) grey lethal gene in the rat); nor does it seem that *S–s* can be a single genetic unit. In *Primula viscosa* Ernst (1936) has discovered allelomorphs of the *S–s* gene which give rise to anomalous combinations of the sub-characters. He has obtained plants in which female (stigmatic) and male (anther) characters has been reassociated to give self-compatible homostyly. He even records plants in which the pollen shows

a reassociation of size and incompatibility characteristics. Homostyly of this kind has also been found in the primrose by Crosby (1940). These various allelomorphs of the *S–s* gene can be more simply understood in terms of three sub-genes which together constitute the gene. One sub-gene may be supposed to control the male characteristics, a second the female characteristics and a third the relation of size and incompatibility in the pollen. If tightly linked, such sub-genes would normally be transmitted as a unit, but rare crossing over could lead to the observed recombinations. Thus breakdown of the genetical switching mechanism would lead to breakdown of the developmental association.

Heterostyly in *Primula sinensis*

In *P. sinensis* heterostyly corresponds in its normal expression and in its normal genetical switching with that of other species of *Primula*. Its observed breakdown, however, has been different from that which Ernst describes in *P. viscosa*.

In the first place, despite the tens of thousands of plants which have been bred during the past fifty years, largely at the John Innes Horticultural Institution, no abnormal allelomorphs of the *S–s* gene have been discovered. While it is true that the great majority of these plants have been pins, which being homozygous (*ss*) offer no opportunity for recombination of sub-genes, it is nevertheless significant that changes of the kind found by Ernst and others have not been observed in the switch gene. There is presumably some difference between the *S–s* gene of *P. sinensis* and that of *P. viscosa*. The difference may not, however, be one of fundamental organization: a small inversion associated with one allelomorph could readily account for the lack of change in the switch gene of *P. sinensis*.

Breakdown of heterostyly has been observed to occur in a different way in the Chinese primrose, viz., by alteration of genes other than *S–s*. Two such genes have been observed to affect heterostyly in a drastic way. One of these is *a* ("Primrose Queen"), a member of a set of four allelomorphs affecting the size of the colored "eye" round the mouth of the corolla tube (De Winton and Haldane, 1933, Mather, 1948). The other three allelomorphs are characteristically associated with the normal expression of heterostyly, even though one of them, a^n, has an effect on the eye which is indistinguishable from that of *a*. It may thus be that the effect on heterostyly is genetically separable from the effect on the eye: that there are really two closely linked genes, one affecting the eye and one heterostyly, and that these have changed simultaneously in *a*, only one of them having changed in a^n. On this view it should be possible to find an allelomorph which effects heterostyly but not the eye. Such a discovery has yet to be made; but it should be remembered that alteration of the eye is much more striking than an alteration of heterostyly. The missing allelomorph, should it be in fact a possibility, would be more likely to escape detection than would *a* and a^n. The effect of *a* on heterostyly will be discussed below.

The second gene which affects heterostyly is *m* ("fertile double") which also gives doubleness of the corolla (De Winton and Haldane, 1933). No allelomorphs other than *m* and its normal alternative, *M*, have been found. The expression of *m* in causing doubleness of the corolla is very variable, much of the modification being genetic. Sometimes not all of the petals are double. The effect on heterostyly is then confined to the anthers associated with the double petals. Thus the two effects of *m* seem unlikely to be genetically separable: they seem to be due to a developmental association. They may, of course, be nevertheless capable of independent genetical modification.

The effects of *a* and *m* on the expression of heterostyly are different, *a* affecting only the position of the stigma, at least to any great extent, and *m* only the position of the anthers.

Since *S* is dominant to *s*, *A'* (and indeed also *A* and a^n in respect of the effect on the style) to *a*, and *M* to *m*, eight phenotypically distinct combinations of these genes are possible. These eight combinations have been produced and measurements made of the position of the stigma, position of anthers, size of stigmatic papillae, size of pollen grain, and speed of growth of pollen tubes associated with each. With the exception of stigma and anther positions, these measurements were made by Mr. B. Misro, whose results, however, are unfortunately in the main not available for discussion. Thus attention must be concentrated on the measurements of stigma position and anther position, though a few of the other observations will be mentioned.

The Effects of "Primrose Queen" and "fertile double" on Heterostyly

Two measurements, X and Y, were made on plants of all combinations of the genes *S-s*, *A-a*, and *M-m*. A third, Z, has been added in the case of *m* plants. The nature of these measurements is illustrated in figure 1. X is the height of the top of stigma from the insertion of the style on the capsule, and Y is the height of the insertion of the anthers above the bottom edge of the corolla tube. The insertion of the anthers is normally marked by a somewhat constricted ring in the corolla tube, but in *m* plants, where the anthers are raised in position, the ring is not corespondingly affected. Thus in *m* plants the third measurement, Z, is the height of the ring above the bottom edge of the corolla tube. Z presumably shows the height at which the anthers would have been inserted if *m* had not in fact led to their being raised.

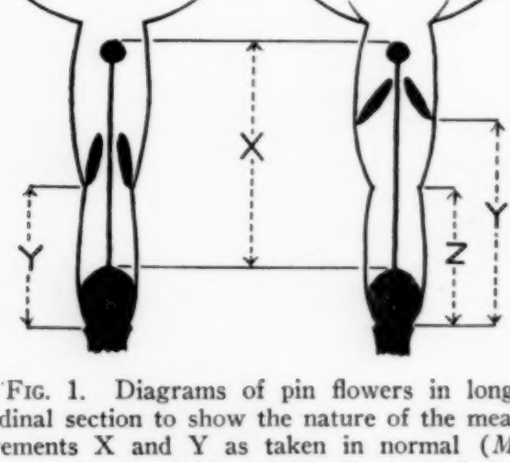


Fig. 1. Diagrams of pin flowers in longitudinal section to show the nature of the measurements X and Y as taken in normal (*M*) flowers (on the left) and "fertile double" (*m*) flowers (on the right). The measurement Z is also taken in *m* flowers. For further description see in the text.

The average values observed for X, Y and Z in plants of the eight genetic constitutions are shown in table 1 (A and B). Generally two flowers were measured from each plant, and two or more plants from a family. The family mean was then found, and the family means were averaged to give the figure shown in the table. This course was adopted because, as we shall see later, different families of a given type in respect of *S-s*, *A-a* and *M-m* may show small differences in their mean values. Measurements were taken during the years 1946, 1947 and 1948, and the total number of families used in each class are given in brackets in table 1A.

The effect of the difference between *S* and *s* is clearly shown in all the four classes distinguished by *A-a* and *M-m*. *S* plants always have anthers higher up and stigmata lower down than the corresponding *s* individuals. In the *AM* group, *S* plants have an X just over half as big as that of the *s* plants, and *s* plants have a Y just over half as big as *S*. The X's of *S* and *s* plants bear much the same ratio to one another in *Am* as in *AM*, but the *S-s* difference in X is very much smaller in the *a* classes. Similarly the corresponding Y ratio is much the same in *aM* plants as in *AM*, but is smaller in the *m* classes.

These effects are clearly related to the main actions of *a* and *m* on heterostyly. These actions have been expressed by the ratio on the right and lower margins of the table. The ratios on the right margin are the mean X (or Y) of the *a* class divided by the mean X (or Y) of the *A* class, and along the bottom are correspondingly the mean X (or Y) of the *m*

TABLE 1A. *Mean heights of stigmata (X) in plants of the eight combinations of S-s, A-a and M-m. In brackets are the numbers of families used in finding the averages. For explanation of the Ratios see in the text.*

Genes		A	a	Ratio
M	S	5.17(12)	3.59(8)	0.69
	s	9.71(36)	4.42(26)	0.46
m	S	4.04(2)	3.33(11)	0.82
	s	9.81(18)	4.44(17)	0.45
Ratio	S	0.78	0.93	
	s	1.01	1.00	

Measurements in m.m.s.

TABLE 1B. *Mean heights of anthers (Y) in plants of the eight combinations of S-s, A-a and M-m. Mean values of Z are given in brackets for m plants. For explanation of the Ratios see in the text.*

Genes		A	a	Ratio
M	S	8.91	9.41	1.06
	s	5.12	5.31	1.04
m	S	10.75(9.21)	11.61(9.50)	1.08(1.03)
	s	7.68(5.06)	8.42(5.66)	1.10(1.12)
Ratio	S	1.21(1.03)	1.23(1.01)	
	s	1.50(0.99)	1.59(1.07)	

Measurements in m.m.s.

class divided by the mean X (or Y) of the M class. Thus for *SM* plants the X ratio of 0.69 in the right margin in 3.59, i.e., mean of X in *SaM* plants, divided by 5.17, i.e., mean of X in *SAM* plants. It will be seen that *a* very materially shortens the style as compared with *A* (right margin of table 1A). Equally *m* raises the anthers as compared with *M* (lower margin at table 1B). The Z values are given in brackets after the Y values for the *m* classes. These are clearly not affected to any extent by the *M-m* difference. The anther insertions are raised by *m*, but the ring remains to mark the position of normal insertion.

It will equally be seen that *m* has no consistent effect on the value of X, i.e., on the height of the stigma (lower margin of table 1A). There is, however, a hint of an effect of *a* on the anthers (right margin of table 1B). The ratio of Y between *a* and *A* plants is always slightly larger than 1. Thus *a* may slightly raise the anthers as well as lowering the stigma, but, even if real, this effect on the anthers is very much smaller than the effect on the position of the stigma. The main effect of *a* is on the female side and of *m* on the male side.

It will be observed that the reduction effected by *a* in X is proportionately greater in *s* than in *S* plants (ratios of 0.46 and 0.45 against 0.69 and 0.82). Similarly the increase effected by *m* in Y is greater in *s* than in *S* plants. This difference in the action of *m* might be accounted for by supposing that *m* did not act by adding a proportionate increment to Y, but by adding a constant increment. Since Y is smaller in *s* than in *S* plants, the effect measured as a ratio would then appear larger. Comparison of the actual measurements, however, suggests that the increment added is in fact greater in *s* than in *S* plants. In any case a similar argument could not apply to the action of *a* on X. Reduction by constant increment would lead to the ratio being lower in *S* than in *s* plants, i.e., to the opposite of that observed.

The effects of *a* and *m* are further illustrated in fig. 2 and table 2. The figure shows diagrammatically the position of anthers and stigmata in the flowers, while the table gives the ratio X/Y for each class. This ratio summarizes the situation of each class in respect of heterostyly. Thus a normal pin has a ratio of nearly 2 (i.e., stigma nearly twice as high as anther insertion) and a normal thrum a ratio of about ½ (i.e., anther insertion nearly twice as high as the stigma). A homostyle will, of course, have a ratio of about 1. So in the pin (*s*) series *aM* plants and *Am* plants approach homostyly, that in *aM* being short homostyly due to shortening of the style and that in *Am* being long homostyly due to raising of the anthers.

When *a* and *m* are combined in an individual, both the shortening of the style and

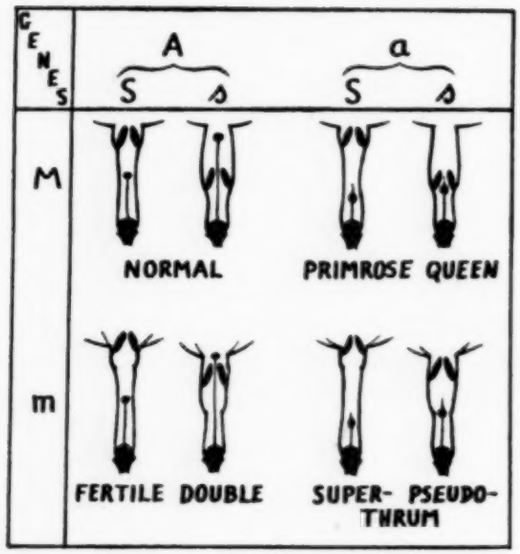


FIG. 2. Diagrams of flowers in longitudinal section to show the position of anthers and stigmata in plants of the eight constitutions in respect of *S–s*, *A–a* and *M–m* (after Mather, 1948).

raising of the anthers will be expected. The result, as will be seen from the table, is that an *sam* plant has its heterostyly reversed. Its stigma is lower than its anther insertion and indeed it is thrum-like with an X/Y ratio of 0.52 instead of the 1.90 for a normal pin. Such *s* plants have been termed *pseudo-thrums* (Mather, 1948).

The effects of *a* and *m* are both in the thrum direction, the one on the stigmata and the other on the anthers. Combined they turn a pin into a pseudo-thrum. They will therefore serve to exaggerate the normal characteristics of thrum plants. The X/Y ratio falls below the 0.57 of the normal thrum in both *SAm* and *SaM* plants. It is reduced even more in *Sam* individuals which have therefore been described as *super-thrum* (Mather, 1948), having the anther inserted nearly four times as high up as the level of the stigma.

TABLE 2. *Mean values of* $\frac{X}{Y}$ *in plants of the eight combinations of S-s, A-a and M-m. The mean values of* $\frac{X}{Z}$ *are given in brackets for m plants. For explanation of the Ratios see in the text.*

Genes		A	a	Ratio
M	S	0.57	0.37	0.65
	s	1.90	0.83	0.44
m	S	0.38(0.44)	0.29(0.34)	0.76
	s	1.26(1.80)	0.52(0.78)	0.41
Ratio	S	0.66	0.78	
	s	0.67	0.68	

The ratios given in the margins of table 2 were found from the X/Y values just as those in the margins of table 1 were found from the X and Y values. These ratios serve to emphasize the difference in effect of *a* in *S* and *s* plants.

While Mr. Misro's more extensive observations on the way in which *a* and *m* affect the other sub-characters of heterostyly are not available, some of his earlier measurements can be quoted. These are given in table 3. They compare style length (X), anther position (Y), mean size of stigmatic papillae, mean size of pollen grain, and growth of pollen tubes in thrum (*SAM*), pin (*sAM*) and pseudo-thrum (*sam*) plants. The range of material measured is much smaller than that upon which table 1 is based, so that the means of X and Y do not correspond exactly with the values in that table.

The stigmatic papillae and pollen grains were measured in arbitrary units by means of a micrometer eyepiece. The growth of the pollen is expressed as the proportion of the length of the style penetrated by the average of the five leading pollen tubes. Cut flowers were pollinated and kept in water in an incubator at 25° C for a constant length of time for these observations.

The positions of stigmata and anther agree tolerably well with those recorded in table 1. It will be seen that the stigmatic papillae of pin flowers are larger than those of thrums and that those of pseudo-thrums agree better with thrums than with pins. Evidently *a* affects the papillae as well as the height of the stigma. The reverse seems to be true of *m*. The pollen of pseudo-thrums agrees in size better with pin than with thrum, so that *m*, while altering the position of the an-

TABLE 3. *Comparison of thrum (SAM), pin (sAM) and pseudo-thrum (sam) plants*

	Height of stigma	Size of stigmatic papillae	Thrum	Pin	Pseudo-thrum
			as male		
Height of anthers			9.80	6.00	8.54
Size of pollen			0.23	0.16	0.17
Thrum (as female)	4.92	0.12	0.65	0.83	0.94
Pin (as female)	10.46	0.15	0.90	0.80	0.72
Pseudo-thrum (as female)	4.90	0.10	0.60	0.70	0.56

The lower left section of the table gives the stigmatic measurements of the three types. The upper right section gives the anther and pollen measurements. The lower right section gives pollen tube growth in all combinations.

Anther and stigma height in m.m.s. Pollen and papilla size in arbitrary units. Pollen tube growth as the proportion of the style penetrated by the leading pollen tubes. Measurements by Mr. B. Misro.

thers, appears to have little if any effect on the size of pollen. Beale's (1939) statement that *m* affects the size of the pollen is not confirmed.

The data on pollen tube growth are not easy to interpret. There is evidence of the incompatibility mechanism between pins and thrums, for the legitimate crosses (pin × thrum and thrum × pin) show better pollen growth than the illegitimates (pin × pin and thrum × thrum). The incompatibility mechanism is, however, not strong, as indeed Mather and De Winton (1941) concluded from earlier observations using Darwin's method of comparative seed setting. It will be recalled that these authors attributed the observed decline in incompatibility within the last fifty years or so to selection by horticulturalists for self-compatibility in pin plants. The present data agree well with this view. The pins show better pollen growth with their own pollen than do the thrums, which are less favored by horticulturalists and which have therefore been subjected less directly to selection.

The self-pollination of pseudo-thrums shows evidence of incompatibility as strong as that of thrums. Pseudo-thrums, however, appear cross compatible with thrums when used as males though not when used as females. Cross-compatibility is intermediate with pins whichever way the cross is made. In general, the pseudo-thrums appear to behave more like thrums as females but somewhat more like pins as males. This agrees with the observations of the effects of *a* and *m* on stigmata papillae and pollen size, as noted above. It also agrees with Beale's (1939) findings, obtained from the study of competition between pollen from different types of plant after double pollination. He found that pins carrying *a* had moved at least some way towards thrums in their stylar reactions, while pins carrying *m* departed little if any from pins in their pollen behavior.

THE EFFECTS OF GENES OTHER THAN *a* AND *m*

The two genes *a* and *m* have drastic effects on the manifestation of heterostyly. No other genes have been discovered with action equal to these, but evidence is nevertheless available that other genes do affect the positions of stigma and anthers.

A number of different lines of pin (*sAM*) have been maintained for several years and measurements X and Y were obtained from 12 of these lines in each of the years 1946, 1947 and 1948. The flowers of these lines obviously differed in size, so that it was deemed advisable to consider not the variation in the absolute heights of stigmata (X) and anthers (Y), but that in the relative heights of stigmata and anthers as measured by the ratio

X/Y. This is the ratio already used in table 2. The mean value of X/Y for each line in each year is given in table 4.

TABLE 4. *Variation in* $\frac{X}{Y}$ *between lines of sAM plants*

Line	1946	1947	1948	Mean	*Ch* v. *ch*
29–27	2.18	2.25	2.27	2.23	*ch*
24–23	2.25	2.19	2.21	2.22	*ch*
2	2.09	2.15	2.02	2.09	*ch*
25–24	1.81	2.11	2.00	1.97	*ch*
5	1.99	1.94	1.89	1.94	*ch*
3	1.83	1.85	1.88	1.85	*ch*
12	1.73	1.96	1.77	1.82	*Ch*
26–25	1.68	1.80	1.99	1.82	*Ch*
27–26	1.84	1.72	1.87	1.81	*ch*
11	1.62	1.84	1.83	1.76	*Ch*
21–20	1.69	1.74	1.77	1.73	*Ch*
8	1.59	1.69	1.40	1.49	*Ch*
Mean	1.86	1.94	1.91	1.90	

It will be seen from table 4 that there is little difference between the grand averages of all lines in the three years. The season appears to have little effect. The lines themselves, however, differ markedly—there being a range in the line averages (taken over years) from 2.23 in line 29–27 to 1.49 in line 8. An analysis of variance confirms these conclusions (table 5).

TABLE 5. *Analysis of variance of* $\frac{X}{Y}$ *in sAM lines*

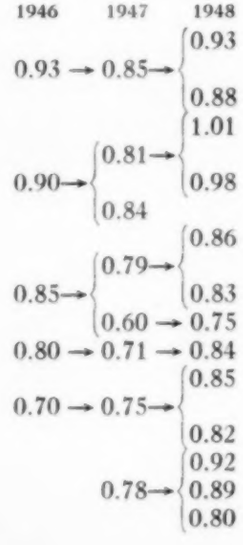
Item	Degrees of freedom	Mean square	Variance ratio	Probability
Years (R)	2	0.0189	2.1	0.20–0.05
Lines (L)	11	0.1194	13.2	<0.001
Error (R×L)	22	0.0090		

These lines differ in a number of the mutant genes known in *P. sinensis*. One of these seems to be associated with the variation of X/Y. This is the gene *Ch–ch* which affects the shape of the corolla, *ChCh* and *Chch* giving the "sinensis" type and *chch* giving the "stellata" type (De Winton and Haldane, 1933). The seven stellata lines (see table 4) give an average of 2.02 and the sinensis line an average of 1.73. The difference between these types is highly significant with a mean square of 0.6675 as compared with the error variance of 0.00902. The remaining 10 degrees of freedom between lines are, however, still associated with a sum of squares of 0.6460, giving a mean square of 0.0646 and a variance ratio of 7.16. For 10 degrees of freedom against 22 this variance ratio is also highly significant with a probability of much less than 0.001.

None of the other major genic differences seems to be associated with the variation in X/Y. Other genes of small effect, presumably members of a polygenic system, would appear to be responsible for this residual variation between lines, which, it will be observed, shows no obvious discontinuity except possibly between lines 24–23 and 2, and between 20 and 8. The difference associated with *Ch–ch* could, of course, also be due to a separate gene linked with *Ch–ch;* but the main action of *Ch–ch* on the shape of the corolla strongly suggests that the difference in effect on X/Y is an outcome of the same initial action.

Similar evidence of the action of genes other than *a* and *m* is to be found in the classes *saM* and *SaM*. The former class derives from a number of different fami-

TABLE 6. *Variation of* $\frac{X}{Y}$ *between lines of saM plants*



lies in 1946. These were represented by descendants in 1947 and 1948 as shown in table 6, in which the averages of X/Y are also given for the various families in the different years. From this table 20 pairs of families can be recognized standing in the parent-offspring relation. The parent-offspring correlation is 0.575 which when based on 20 independent sets of parent and offspring has a probability of just about 0.01. The independence of certain sets of observations is, however, doubtful: in several cases different families are the offspring of the same parental family. We have in fact 12 parent families, so that we should perhaps regard 0.575 as an estimate of the correlation of 12 sets of parent and offspring, weighted by the number of families each parent family has produced. For 12 sets of observations the probability is 0.05. The evidence for differences between the lines is thus less strong, but is still not to be neglected.

All but one of the *SaM* families fall into two lines originating from separate families in 1946 (table 7). An analysis of variance into variation between and within these lines gives mean squares of 0.003601 for 1 degree of freedom between lines, and 0.000548 for 5 degrees of freedom within. Thus $t_{[5]} = 2.563$ with a probability of 0.05.

TABLE 7. *Variation of* $\frac{X}{Y}$ *between lines of SaM plants*

1946	1947	1948
0.37	→ 0.39	→ 0.42
0.34	→ 0.32	→ 0.37
		→ 0.36

There is thus suggestive evidence of differences between the lines in both the *saM* and the *SaM* classes. The results from the other classes did not lend themselves to any such test. Thus of the three classes (*sAM*, *saM* and *SaM*) where tests for differences between lines could be carried out, one gives clear evidence and two very suggestive evidence of such differences. Clearly genes other than *A–a* and *M–m* affect the heterostyly normally switched by *S–s*. These other genes have relatively small effects and so reveal to us a means whereby the precise expression of heterostyly could be adjusted within fine limits, apart from the more drastic effects of *A–a* and *M–m*.

Variation in Heterostyly within the Plants

A further effect of genes other than *S–s*, *A–a* and *M–m* came to light from the crossing of three lines of pin plants. Two of these lines had been inbred by some thirty generations of self-pollination, and the third by rather more than ten generations, before the experiment began. Two of them were *sAM* and the third *sAm* in constitution.

The lines were crossed together and the three F_1's raised from them. No attention will be paid to the direction of the cross, as the available evidence fails to show any difference between reciprocals. F_2's were raised from the F_1's.

Parents, F_1's and F_2's were grown in 1946, parents and F_2's again in 1947 and parents and F_1's in 1948. The measurements of X, Y and (where appropriate) Z were made on two flowers of each plant in the various generations of each year. The variance of X was analyzed into items between plants and between flowers within plants. Where different families of the same line, F_1 or F_2 were grown in different parts of the greenhouse an item was taken out during analysis to represent the variation due to difference between families or sites. This item of the analysis will be considered no further because, taking the experiment as a whole, no evidence could be found of differences between families or sites greater than the variation between plants within families or sites.

The mean square differences in X between plants and within plants (i.e., between flowers) are given in table 8. They are shown for the different lines and crosses separately and are also averaged

TABLE 8. *Variation in X within plants (F) and between plants (P) measured as mean squares* (In brackets are the numbers of degrees of freedom upon which the mean squares are based.)

		F 1946	P	F 1947	P	F 1948	P
Parent lines	A(*sAM*)	0.26(16)	1.09(14)	1.10(22)	1.11(18)	0.43(54)	1.07(48)
	B(*sAm*)	0.56(11)	0.76(8)	0.57(23)	0.66(20)	0.38(43)	0.75(39)
	C(*sAM*)	0.25(40)	0.60(36)	0.13(24)	0.82(20)	0.42(44)	0.87(35)
Average		0.36	0.82	0.60	0.86	0.41	0.89
F_1	A×B	0.08(25)	0.13(23)	—	—	0.24(13)	1.80(12)
	A×C	0.17(27)	0.32(21)	—	—	0.23(56)	0.18(52)
	B×C	0.13(25)	0.09(20)	—	—	0.16(11)	0.49(8)
Average		0.12	0.18	—	—	0.21	0.82
F_2	A×B	0.33(36)	1.86(35)	0.21(48)	3.85(46)	—	—
	A×C	0.24(40)	3.36(38)	—	—	—	—
	B×C	0.29(21)	2.18(20)	0.15(27)	1.31(24)	—	—
Average		0.29	2.47	0.18	2.58	—	—

over parental lines F_1's and F_2's for the various years.

The mean squares between flowers (F) are generally lower than those between plants (P). In the case of the F_2's, F is very much lower than P, which is itself much higher in F_2 than in either parents or F_1. Thus there is segregation for genes affecting X, even though none of the major genes which affects X is segregating. Since the F_2's show segregation the original lines must have differed in such genes, just as was found in the experiments described in the last section. The P values for parents and F_1 are not nearly so much larger than the F values as they are in F_2. This smaller excess of P over F can reasonably be ascribed to the small differences of environment which individually potted plants must be expected to experience. This conclusion need in no way conflict with the apparent absence of differences due to site in the greenhouse, for the environment differences between one pot and another can include, for example, differences in fertilizer and water application which are not necessarily related to position in the greenhouse.

The chief interest of table 8, however, lies in the mean squares between flowers of the same plants. F is greater in the parental lines than in the F_1's. The F_2's lie between the parents and the F_1's, nearer to the latter. Differences in style length between flowers of the same plant must be attributed to differences in development, and it thus appears that the parental lines are subject to greater developmental variation in style length than their F_1's or even F_2's.

Table 8 refers only to X, the height of the stigma. Data are available, however, for anther height in certain of the families of 1946. This was measured by Y in *M* plants. In *m* plants, whose anthers are higher than normal, Z, the height of the ring, has been used in place of Y. In this way *m* plants have been incorporated into the analysis of F_2, and line B has been brought into the analysis of the parents. Table 9 gives the average of mean squares

TABLE 9. *Variation in X and Y within plants (F) and between plants (P) measured as mean squares*

	X		Y	
	F	P	F	P
Parent lines	0.29	1.04	0.10	0.43
F_1's	0.12	0.18	0.05	0.09
F_2's	0.29	2.47	0.07	0.23

of X and Y (replaced by Z where necessary) for these families of 1946. It will be seen that developmental variation is less in anther height than in stigma height, perhaps because, being pin, these families have smaller values of Y than of X. It will also be observed that there is little evidence of segregation in F_2 for genes affecting anther position as opposed to style length. The excess of developmental variation in the parent lines as compared with F_1 is, however, observable in anther height as in stigma height.

The main difference between the inbred parental lines and their F_1's is to be found in the former's homozygosity and the latter's heterozygosity. Evidently the homozygous genotypes produced by inbreeding are more subject to developmental variation, less able perhaps to cope with the vagaries and chances of development, than the heterozygous genotypes of F_1. In so far as heterostyly has been successful in the promotion of outbreeding in the ancestors of our cultivated Chinese primroses, the natural genotype, and hence the one which has been balanced by natural selection, must be heterozygous for many of its genes. Thus the increase in developmental variation to be seen in the inbred lines is another form of inbreeding depression.

The sub-characters associated with heterostyly show the effects of genes other than those producing drastic changes, not only in their gross expression but also in the variation of this expression. They show the effects of these genes by segregation in F_2 and also by the manifestation of a kind of inbreeding depression, or heterosis if we choose to look at it the other way. That the inbreeding depression is shown by the character which normally curtails inbreeding is of interest, but it is not to be regarded as remarkable unless we are prepared to regard characters controlling the breeding system of a species as subject to rules of genetical adjustment different from those governing other characters of size and shape. No evidence has yet come to hand in support of such a distinction; and indeed evidence, such as the above, is available against it.

The Evolution of Heterostyly

Three types of genic effect on heterostyly can be recognized in *P. sinensis*. First of all there is the action of *S–s*, the switch gene whose segregation is responsible for the normal variation upon which depends the operation of heterostyly as an outbreeding mechanism. The other two types of gene contribute to the genetic background in the sense that their variation is not a necessary part of heterostyly, and indeed, with some of them, may destroy it. Of these two types, one includes the genes *A–a* and *M–m*, whose variation can so alter heterostyly as to replace it by homostyly, or, when acting together, can even bring about what is in morphological respects a new pin-thrum difference inside the class homogeneous for the pin genotype in respect of *S–s*. The third type includes those remaining genes which can be shown to affect the expression of heterostyly but whose effects are sufficiently small for them to be regarded more as the agents of fine adjustment than of essential structure.

The effect of *Ch–ch* is sufficiently large for the legitimacy of its inclusion in this last class to be questioned. But the other differences in mean expression among the various pin lines described in the preceding two Sections and in developmental variation as described in the last Section, would seem to be small enough to be properly regarded as the potential material for close adjustment to needs.

These conclusions are based chiefly on study of the morphological sub-characters of heterostyly, particularly anther and stigma height. Attempts have been made to investigate the physiological sub-character, incompatibility, by the observation of pollen tube growth and by the comparison of seed setting following various types of pollination. Some of Misro's observations on pollen tube growth and Beale's observations on competitive pol-

lination have been mentioned. Little other evidence is available. Mather and De Winton have pointed out how the efficacy of incompatibility as an outbreeding mechanism has declined as a result of horticultural selection. This decline has occurred even though the switching gene *S–s* still switches such incompatibility as remains, and even though morphologically the plants are still good pins and thrums. Again it seems that genes must be postulated as controlling the background in such a way as to determine the precise nature and efficiency of the incompatibility which *S–s* switches. They are genes of adjustment similar to those recognized from the above study of the morphological sub-characters. Both sets of genes seem likely to operate in polygenic systems.

In spite of extensive experiments on comparative seed setting it has been impossible to press the genic analysis of incompatibility any further. The results of these experiments have been too much disturbed by disease and by the effects of environmental upset for them to be regarded as reliable.

Our knowledge of the detailed genetical control of heterostyly is thus by no means complete. It is, however, already clear that the expression of heterostyly is variable in all the sub-characters that have been tested, morphological and physiological. And in so far as it is variable, we must suppose (as would indeed be likely on general grounds) that the level of expression has been adjusted by natural selection in the wild ancestors of our cultivated Chinese primrose. The display of inbreeding depression in the form of increased developmental variability adds emphasis to this conclusion.

This adjustment must have proceeded, at least to some extent, independently in the various sub-characters. The effects, for example, of the genes *A–a* and *M–m* in *P. sinensis*, and of the aberrant allelomorphs of *S–s* in other species, show that the sub-characters are not all bound indissolubly together. Their regular developmental association in the alternative pin and thrum forms must therefore arise from control by a background genotype which has been adjusted by natural selection to give such regular behavior; when by mutation or selection we depart from this genotype the sub-characters become partially dissociated in development. The unitary behavior of heterostyly has been achieved by selection of the genotype, not imposed by the restrictions of development.

Thus adjustment of the genetical background gives heterostyly its special character; a character which, as we now see, can have been built up gradually with gradually increasing efficiency, by the accumulation, under the action of selection, of the balanced set of genes upon which it depends. When we destroy this balance we destroy heterostyly, even though the gene *S–s* still continues by its segregation to switch two alternative, but now no longer mutually adapted, forms. The evolution of heterostyly must, in one of its aspects, have been the evolution of this genetic background.

In *P. sinensis* we have no information about the organization of the switching gene itself though we may suspect that a gene with its special properties is hardly likely to be a fortuitous occurrence. From the work of Ernst and others on other species of *Primula* it is, however, clear that *S–s* is itself composite. Three parts, or sub-genes, have already been recognized and these appear to be recombinable in the sense that new allelomorphs can be found leading to reassociations of the sub-characters of heterostyly. The essential unity of the gene would thus appear to be due more to close linkage and rare recombination than to any indissolubility of the gene in its overall action.

The present observations on *A–a* and *M–m* in *P. sinensis* strengthen this conclusion. The one affects the style and the other the anthers in much the same way as do the parts which Ernst has recognized in *S–s* elsewhere. The pseudo-thrum, *sam*, is not a simple alternative to

pin, *sAM*, in *P. sinensis*. But it fails to be a simple alternative only because *A–a* and *M–m* segregate independently. If *A* were closely linked with *M* and *a* with *m*, they would jointly constitute a functional switch gene alternative to *S–s* at least in respect of the gross morphological sub-characters of heterostyly. It is true that *A–a* and *M–m* have other effects as well, but we can hardly doubt that, provided they fulfilled the segregational requirements of a switch gene, these other effects could be adjusted and removed by the selection of modifying genes. *S–s* is unique only in the essential linkage of its parts, a linkage which *A–a* and *M–m* do not share.

We can thus see that in its role as the organized switching agent of heterostyly, the *S–s* gene is itself the product of selection. The raw materials from which a switching system can be built may exist, unorganized and therefore incapable of working, in the genotype. Should these materials happen to arise together, or to be brought together by structural change, the integrated switching becomes possible. Once together they may continue to reveal their origin by occasional recombination and breakdown, or, as may well have happened in *P. sinensis*, become tied up once and for all into a super-gene by a small inversion, or other means of suppressing recombination.

There is thus no conflict, in relation to the origin of heterostyly, between the findings of Ernst in respect of the switch gene and the observations on the function of the genetic background in *P. sinensis*. Rather the two reinforce one another. Heterostyly depends on an adequately adjusted genetical background and on an efficient switch gene. Both are the product of natural selection acting on genic raw materials which can be traced in *P. sinensis*. Selection can be seen as having balanced up the various genes which constitute the background, and as having picked out that mechanical integration upon which depends the efficient working of the switch gene. Where and how there originated the genic structures whose balance is adjusted in the one case or whose mechanical integration is achieved in the other, is a question into which we have as yet no means of enquiring. We can hardly doubt, however, that they themselves are the product of some still deeper process of genic evolution.

Summary

Heterostyly is a complex character involving six sub-characters, morphological and physiological. Its normal operation is controlled by the segregation of a switch gene comprising two allelomorphs, *S* (giving thrum) and *s* (giving pin).

Additional allelomorphs of the switch gene have been found by Ernst and others in *Primula* species, but no such variation of *S–s* has been observed in *P. sinensis*.

In *P. sinensis* the recessive gene *a*, giving "Primrose Queen" eye, also shortens the style, so that an *aa* pin is a short homostyle. The recessive gene *m*, giving "fertile double" flowers, raises the anthers so that an *mm* pin is a long homostyle. The effects of *a* on eye size and style length may be genetically separable by mutation but those of *m* on corolla and anthers appear not to be so. They may nevertheless be separably modifiable. It is possible that *a* may also raise the anthers slightly, but *m* has no consistent effect on the style. The effect of *a* is proportionately greater in pin than in thrum plants. A "Primrose Queen, fertile double" pin (*ss aa mm*) resembles a normal thrum (*SAM*) in the positions of anthers and stigma. It is a pseudo-thrum. *Sam* is more extreme than normal thrum and is described as a super-thrum.

The gene *a* affects the size of stigmatic papillae as well as style length, but *m* appears not to affect the normal pin-thrum difference in pollen size. The incompatibility reaction between pin and thrum has been weakened, but not eradicated, by horticultural selection in *P. sinensis*. *a* appears to affect the incompatibility reaction of the style, moving it in pseudo-thrum plants part way towards thrum be-

havior; but *m* has little if any effect on the incompatibility properties of pollen.

Genes other than *s*, *a* and *m* affect the relative positions of anthers and stigma in *sAM*, and (most probably) *saM* and *SaM* flowers. The gene *Ch–ch* (sinensis-stellata flowers) has a fairly large effect, but other gene differences of smaller effect must also be involved. Inbred lines of pin plants show greater differences between flowers of the same plant in style length and anther height than do their F_1's. Such increased developmental variation is thus a form of inbreeding depression. It would not be expected in wild plants where heterostyly enforces an outbred genotypic balance.

The evolution of heterostyly must have involved the production of a background genotype balanced by natural selection to give the coordinated complex of characters whose alternatives together constitute heterostyly. The sub-characters of heterostyly are at least in part separable in development and must therefore have been adjusted at least partly independently in this balancing process. The variation, particularly the smaller genic effects, which has been revealed in the background genotype would supply the raw genetic material for the gradual rise and ultimate fine adjustment of the heterostyly character.

The switch gene itself is composite, as shown by Ernst's observations. The genes *a* and *m* show that genes with the properties of the sub-genes of *S–s* can exist unorganized in the genotype. The *S–s* difference must therefore have been selected for the mechanical integration of its parts. Structural change may have played a role in this process, and inversion may be responsible for the final tying together of the *S–s* difference into a super-gene in *P. sinensis*.

The experimental work described above was carried out at the John Innes Horticultural Institution, to whose Director, Dr. C. D. Darlington, I am greatly indebted for permission to make use of it in the preparation of this paper, which is based on an account given to the Sixth International Botanical Congress held in Stockholm during July 1950.

References

Beale, G. H. 1939. Further studies of pollen tube competition in *Primula sinensis*. Ann. Eugenics, **9**: 259–68.

Crosby, J. L. 1940. High proportions of homostyle plants in populations of *Primula vulgaris*. Nature, **145**: 672.

Darwin, C. R. 1877. *The different forms of flowers on plants of the same species*. Murray, London.

De Winton, D. and Haldane, J. B. S. 1933. The genetics of *Primula sinensis*. II. Segregation and interaction of factors in the diploid. J. Genet., **27**: 1–44.

Ernst, A. 1936. Heterostylie-Forschung. Versuche zur genetischen Analyse eines Organisations—und 'Anpassungs'—Merkmales. Z.I.A.V., **71**: 156–230.

Grüneberg, H. 1938. An analysis of the "pleiotropic" effects of a new lethal mutation in the rat (*Mus norvegicus*). Proc. Roy. Soc. B, **125**: 123–43.

Lewis, D. 1949. Incompatibility in flowering plants. Biol. Revs., **24**: 472–96.

Mather, K. 1948. Heterostyly in *Primula sinensis*. Rep. John Innes Hort. Instn. (1947), pp. 10–13.

Mather, K., and De Winton, D. 1941. Adaptation and counter-adaptation of the breeding system in *Primula*. Ann. Bot. N.S., **5**: 297–311.

PIGMENTAL VARIATIONS IN POPULATIONS OF POCKET GOPHERS

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INTRODUCTION

Critical analyses of the differences between populations, races, and species of rodents with respect to pigmental factors, have been made with only a few of our native mammals. Sumner and Swarth (1924) were among the first to be concerned statistically with the color of mammalian pelage and the color of the soils on which they lived. Dice and his coworkers however have been responsible for most of the published work to date (Dice and Blossom, 1937; Dice, 1940, 1941, 1944a, 1944b).

The present report deals with an analysis of the pigmental variations of the Botta pocket gopher (*Thomomys bottae*). This extremely variable species has over 30 recognized sub-species in California alone. It was selected for this investigation because the animal is abundant, easily collected, and because its fossorial nature keeps it in darkness almost all of its life.

ACKNOWLEDGMENTS

The work was made possible by grant number 1064 from the Penrose Fund of the American Philosophical Society. Miss Alice Bell, Fresno State College, advised particularly on the use of the statistics. Dr. Willis Rich, Stanford University, likewise gave valuable assistance and advice. Dr. Lee R. Dice, University of Michigan, gave me constant encouragement and many helpful suggestions on the manuscript.

METHODS

Six stations were selected for collecting, beginning at Fresno, California (elevation 290 feet) and extending more or less along an air line 29 miles northeast to the foothill town of Auberry (elevation 1,950 ft.). In order, these stations are designated from west to east, or from the San Joaquin Valley into the foothills of the Sierra Nevada mountains, as Fresno, College 10 acres, Enterprise, Collins, Kern-Friant and Auberry.

Trapping was done during the winter months from 1947 to 1950 while the animals were in the winter pelage. Only animals known to be adults as shown by size of the gonads, uterine scars, or by a gap in pubic symphysis were saved and made into the conventional study skins. With the exception of the Fresno station the collections were all made on areas of less than five acres at each station. After the skins were cleaned a small area between the shoulders of each one was used for color determination. The reflected red, green, and blue were measured for each skin using a Photovolt photoelectric reflectionmeter, model 610. The readings were all made after carefully setting the instrument according to the directions accompanying it. With this instrument quantitative measurements of the colors were easily obtained and expressed as per cent of the colors reflected from magnesium oxide. Standard errors of means and standard errors of differences between the means were computed by using the formulae for small samples. Differences between two means are expressed as t values (table 1). The population density varied from 20 animals per acre in November to 12 per acre in May on uncultivated hog-wallow land. All of the stations west of Collins lie in cultivated irrigated land and no accurate survey could be made. Impressions based on

TABLE 1. *Showing the values between the various station means for red of dorsal pelage. Double asterisk (**) indicates 0.01 probability or less; single asterisk (*) indicates 0.05 probability or less*

	Auberry	Kern-Friant	Collins	Enterprise	College 10-acres	Fresno
Auberry		1.22	**4.30	**5.00	*2.24	**8.42
Kern-Friant	1.22		*2.54	**3.70	0.96	**6.75
Collins	**4.30	*2.54		1.82	1.41	**5.06
Enterprise	**5.00	**3.70	1.82		**2.81	*2.20
College 10-acres	*2.24	0.96	1.41	**2.81		**5.69
Fresno	**8.42	**6.75	**5.06	*2.20	**5.69	

trapping, however, are that there are considerably less animals per acre on the western stations.

The Auberry station is nearly in the center of the range of *Thomomys botta mewa* and it is assumed that only *mewa* are present there. Likewise the Fresno station is near the center of the range of *Thomomys bottae pascalis*. In order to investigate the population composition of the other stations lying between Auberry and Fresno with respect to these two races and their intergrades a linear discriminant function $z = \lambda_1 \ x_1 + \lambda_2 \ x_2$ (Hoel, 1947) was set up for *mewa* and *pascalis*, for Auberry and Fresno specimens, using as variables the length of the hind foot (x_1) and red pelage color (x_2). The λs in such a function are determined so that the difference between the means of the two races will be maximized relative to the standard deviations within the two races (Kendall, 1948; Fisher, 1936). To use the function, z is calculated for any unallocated specimen, and the specimen is then assigned to one sub-species or the other, accordingly as the calculated z is within the range of values of z for one sub-species or the other. The range of the z for the Auberry population is 0.788 – 0.996 and 1.166 – 1.282 for males and females respectively. The range of z for Fresno is 1.068 – 1.260 and 1.298 – 1.462 for males and females, respectively. Thus for a particular sex there is no over-lapping between the zs of two populations. For example in male pocket gophers λ_1 (hind foot) = 0.030 and λ_2 (dorsal color) = 0.014 or, for males, $z = 0.030\,x_1 + 0.014\,x_2$. Thus a male pocket gopher with a hind foot (x_1) of 27 mm. and red color (x_2) of 12 units would be: $z = 0.030 \times 27. + 0.014 \times 12$, or $z = 0.978$, which falls within the limits of z for the male pocket gophers at Auberry. Therefore this animal would be assigned to the race *mewa*. The length of the hind foot and red pelage color were used here because they are less likely to be correlated with each other than two different pelage colors or any two measurements of length or weight. A summary of the data used is shown (Table 2). The use of the discriminant function in allocating individual animals results in greater accuracy than the use of single characters. For instance, many of the animals at College 10-acres might be identified erroneously as *mewa* if only dorsal color or hind foot were considered separately or together. However, when they are compared with the Fresno and Auberry populations by means of the discriminant function, using both dorsal color and hind foot length, all are allocated as *pascalis*, which their distribution well within the range of *pascalis* would also indicate. The figure and the three tables present the relevant data.

TABLE 2. *Summaries of hindfoot length and dorsal pelage color (mean, standard error, standard deviation, and range)*

		Hind foot length	Dorsal pelage color		
			Red	Green	Blue
Auberry	♂ 14	26.50 ± .73 2.66 (23–29)	8.07 ± .60 3.21 (4–11)	4.86 ± .47 2.50 (3–6)	3.75 ± .47 2.50 (2–5)
	♀ 15	25.86 ± .85 1.75 (25–27)			
Kern-Friant	♂ 11	25.63 ± .90 2.87 (22–27)	9.23 ± .73 3.69 (6–16)	5.15 ± .54 2.70 (4–8)	3.76 ± .29 1.49 (3–5)
	♀ 15	25.00 ± .51 1.91 (23–27)			
Collins	♂ 23	28.56 ± .72 3.42 (26–32)	11.55 ± .55 3.66 (7–18)	6.66 ± .36 2.44 (4–9)	4.80 ± .28 1.90 (3–7)
	♀ 22	25.25 ± .45 2.10 (23–26)			
Enterprise	♂ 10	29.60 ± .76 2.30 (25–32)	13.64 ± .95 4.96 (10–18)	8.32 ± .41 2.93 (6–12)	5.42 ± .98 2.26 (4–8)
	♀ 18	27.50 ± .45 1.92 (25–31)			
College 10-acres	♂ 6	29.66 ± .95 2.14 (26–32)	10.23 ± .75 4.41 (7–16)	7.6 ± .50 2.72 (5–11)	5.54 ± .50 2.73 (3–8)
	♀ 22	29.36 ± .57 2.62 (25–32)			
Fresno	♂ 29	30.89 ± .50 2.65 (29–35)	16.32 ± .77 5.38 (9–25)	9.58 ± .43 3.03 (5–14)	6.50 ± .37 2.58 (4–10)
	♀ 21	28.43 ± .45 1.95 (27–31)			

DISCUSSION

When the College 10-acres is excepted it will be noticed that the means of dorsal red pelage range from lower to higher values as one goes from Auberry to Fresno (table 2). The College 10-acres lies just north of the Fresno-Auberry air line in the deep dark soil of the San Joaquin River Valley. The color reading for this soil for red is about one-third to one-half

TABLE 3. *Colors of surface soil at four stations. Means and extremes of 10 samples from each station are given*

	Red	Green	Blue
College 10-acres	14.1 (6–19)	9.6 (5–12)	7.0 (5–12)
Auberry	20.7 (11–27)	12.8 (7–17)	8.8 (7–12)
Kern-Friant	21.0 (17–29)	12.1 (10–16)	6.7 (5–9)
Fresno	27.2 (23–30)	15.5 (12–20)	10.8 (9–13)

lower than are those for the soils at the other stations (table 3). The red pelage color of the pocket gophers inhabiting College 10-acres is significantly much lower when compared with other pocket gophers of the same sub-species at the next nearest station at Fresno (table 1). With respect to red color in the dorsal pelage the race *pascalis* that lives on the dark humus-filled soil at the College 10-acres station is nearer the pocket gophers belonging to the *mewa* race at Auberry and Friant than it is to its own sub-species (table 2). Dice and Blossom (1937) likewise found the darkest race of *Thomomys bottae* in Arizona living in the humus-filled soils. Dice (1944b), working with *Peromyscus maniculatus* in the laboratory but using stock collected in native habitats, found a similar indication of depth of pelage color to be correlated with heaviness of vegetation of the native habitats.

The pocket gophers in this part of California agree with the surface living mammals of southern Arizona in being lighter (higher color readings) in the desert region (Fresno) and darker in the mountains (Auberry). Dice and Blossom (1937) and Dice (1944b) believe this tendency toward dark pelage color for montane forms to be indirectly re-

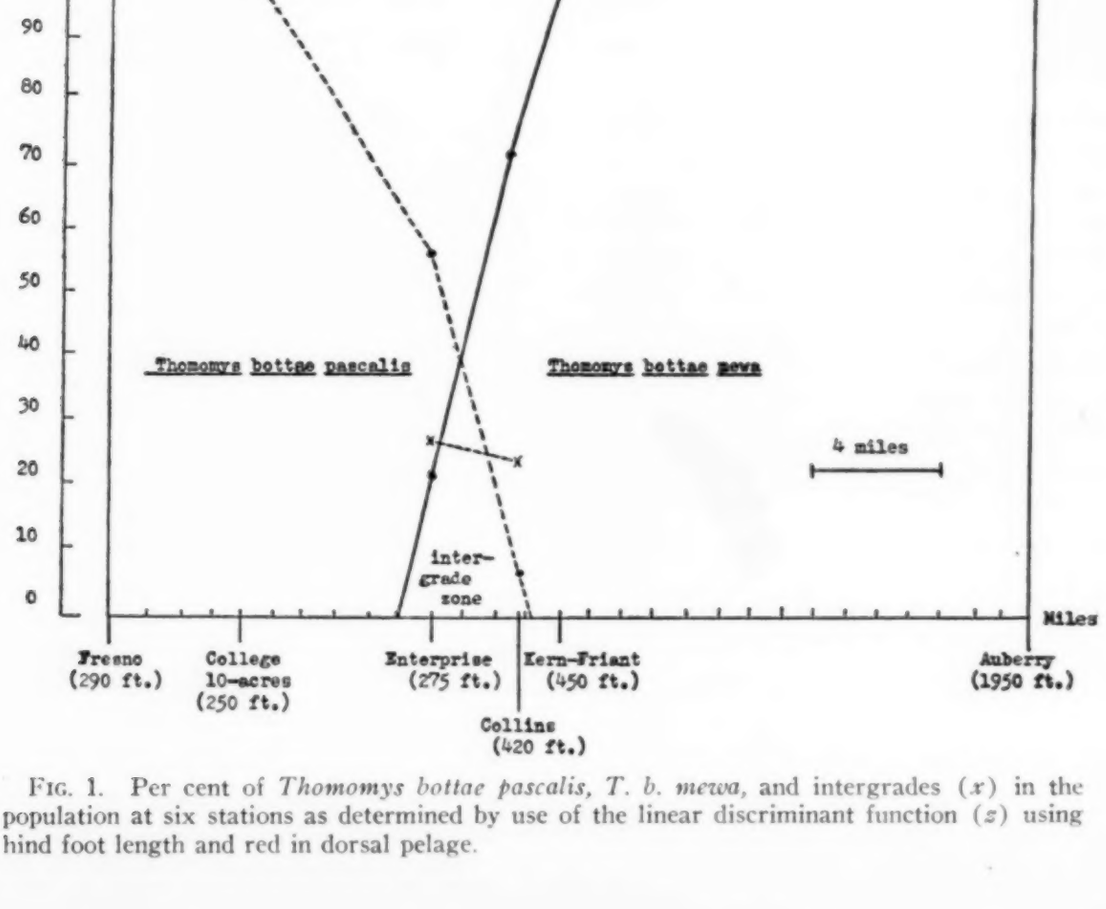

FIG. 1. Per cent of *Thomomys bottae pascalis*, *T. b. mewa*, and intergrades (x) in the population at six stations as determined by use of the linear discriminant function (z) using hind foot length and red in dorsal pelage.

lated to humidity. The heavier precipitation at higher altitudes produces more vegetation, which adds more dark humus to the soil on which darker animals survive and live. The College 10-acre station corroborates this view with dark pocket gophers living in dark humus-filled soil, which is closely flanked (not over a half mile) by dry desert light colored soils (Fresno and Enterprise) with significantly lighter pocket gophers living in them (table 1). The pocket gopher lives in a closed burrow most of its life and it seems would be much less affected directly by variations of atmospheric humidity than animals that live on the surface. There is no evidence that climatic differences in any way directly affect the characters of the pocket gophers.

The per cent of *pascalis*, *mewa*, and their intergrades (as determined by linear discriminant functions) are shown (fig. 1) for each station. The zone of intergradation between the races extends for about four mlies along the Fresno-Auberry air line. This does not mean, however, that the zone is actually that wide for there is nothing to indicate that the intergrading zone and the air line along which collecting was done are at right angles to each other. There are three kinds of animals present within the zone of intergradation; *pascalis* which becomes relatively fewer to the east, *mewa* which becomes fewer to the west, and intergrades which never represent more than 25 per cent of the population at the two places within the zone where collections were made. There is no visible physical reason why this intergrading zone should occur where it does. The San Joaquin Valley gives way suddenly to the Sierra Nevada foothills fully three miles farther east. None of the soil types end in or near the margins of the intergrading zone.

Suspected differences between the population densities at the eastern edge of the *pascalis* range and western edge of the *mewa* range may account for the intergrading zone being where it is today. The fact that *mewa* has a known high density and *pascalis* appears to have a much lower density at the margins of their respective ranges corroborates this view.

Summary

An analysis was made of the pigmental variations occurring in six populations of two adjacent races of *Thomomys bottae* in California. The dark humus-filled soils harbor darker animals than the lighter colored desert type soils. The zone of intergradation at the place where the collecting line crossed it is about four miles wide; however, no data are at present available to show that the line was crossed at right angles. The population of the intergrading zone includes both of the races and not more than 25 per cent intergrades at the two stations tested. No visible physical reason is known why the intergrading zone occurs where it does, though differences in population densities between the contiguous subspecies are suspected.

References Cited

Dice, Lee R. 1940. Ecology and genetic variability within species of *Peromyscus*. Amer. Nat., **74**: 212–221.

——. 1941. Variations in the deer mouse (*Peromyscus maniculatus*) in parts of Oregon, California, and Baja California. Contrib. Lab. Vert. Gen. Univ. Mich., **18**: 1–11.

——. 1944a. Variation of the deer mouse (*Peromyscus maniculatus*) in parts of Idaho, Montana, Utah, and Wyoming. *Ibid.*, **28**: 1–13.

——. 1944b. Variations of the deer mouse (*Peromyscus maniculatus*) in a mountainous area of southern New Mexico. *Ibid.*, **30**: 1–10.

Dice, Lee R., and Philip M. Blossom. 1937. Studies of mammalian ecology in southwestern North America with special attention to the colors of desert mammals. Publ. Carnegie Inst. Wash., **485**: i–iv, 1–129, 8 pls., 8 figs.

Fisher, R. A. 1936. The use of multiple measurements in taxonomic problems. Ann. Eugenics. London, **7**, 179–188.

Hoel, Paul G. 1947. Introduction to mathematical statistics. John Wiley & Sons, Inc., New York. x + 259 pages.

Kendall, M. G. 1948. Advanced theory of statistics. Chas. Griffin and Co. Limited, London. Vol. **2**, vii + 521 pages.

Sumner, Francis B., and Harry S. Swarth. 1924. The supposed effects of color tone of the background upon the coat color of mammals. Journ. Mammal., **5** (2) 81–113; plates 6–12.

NOTES AND COMMENTS

SUPPLEMENTARY NOTES ON *CREPIS*. III. TAPROOT VERSUS RHIZOME IN PHYLOGENY

ERNEST B. BABCOCK

In a recent issue of this journal I proposed a rearrangement of the primitive sections of the genus *Crepis*.[1] This new sequence of sections recognizes the morphologically most primitive group of taprooted species, including *C. pontana* with 5 pairs of chromosomes, as more primitive than any of the rhizomatous species, some of which have 6 pairs of chromosomes. The basis for this change rests (1) on the fact that *C. pontana*, in addition to being taprooted, is just as primitive in other morphological characters as any rhizomatous species; (2) on the conclusion, from my own observations on young seedlings, that the taproot is a more primitive character than the rhizome; (3) on the assumption that, even though no 6-paired, taprooted species of *Crepis* are known, *C. pontana* probably descended from an ancestor (perhaps now extinct) with 6 pairs of chromosomes. Additional light on the chromosome situation awaits further botanical exploration in central Asia. It is with additional evidence on the relative primitiveness of taproot and rhizome that the present note is concerned.

Mattfeld,[2] in a current review of my monograph, includes a discussion of the taproot vs. rhizome question in *Crepis*. He kindly sent me a copy of the galley proof of his lengthy review, the most thorough critique of my work thus far published, and gave permission to publish a translation. The paragraphs devoted to the taproot-rhizome problem follow.

"The direction of a line of development cannot always be determined unequivocally in arranging related forms according to the phylogenetic point of view. In the case of *Crepis* this is especially true for the main arrangement according to form and duration of growth. Primitive are the species which are perennial by means of a rhizome, derivative the species with a lignifying taproot, most reduced the species with an annual taproot. Since the persistence of the primary root and its transformation into a taproot is quite generally something primitive among the dicotyledons, the foregoing arrangement has already become a much debated question (Babcock, in correspondence). The fact that the annual growth form is derivative in the genus *Crepis* seems quite assured through the parallelism of the reduction of the karyotype and the morphological phenotype.

"Babcock designates as rhizome all short stems that have no taproot (horizontal, plagiotropic rhizomes seem to be rare). It would be natural to assume that these (short, vertical rhizomes) have lost the primary root and substituted adventitious roots, thus being derivative. Now the rhizomatous species [actually all of those with plagiotropic, and some of those with vertical rhizomes—E. B. B.] are primitive in their other morphological characters and show many resemblances to the primitive Central-Asiatic genera of the Crepidinae which are rhizomatous, especially *Dubyaea*, from which *Crepis* must be held as derived. [Only some *Dubyaea* species are rhizomatous, others are taprooted—E. B. B.]. Hence the rhizome is considered to be primitive in *Crepis* and its immediate ancestors; only in the generations lying farther back it developed from the perennial taproot. Later on, in adaptation to drier habitats, a taproot was again evolved within *Crepis* through mutations.

"The phylogenetic system of *Crepis* depends on the decision of this question. The referee's own experiments with seedlings (20 species of all types of growth in 58 samples) in the spring of 1949 produced, as expected, the result that all the species developed with a strong vertical primary root after the fashion of a taproot; even by the end of September this taproot had not died off in any one of the species.

"The annual and biennial species do not form any adventitious roots on the epicotyl; the main root develops quickly and strongly, it may be branching, but for the most part it forms only fibrous roots. Among the perennial species examined, those with a taproot were *alpestris, conyzaefolia, pannonica* and *pontana;* those with a rhizome, *aurea, Jacquini* and *sibirica*. The behavior of the primary root varies in almost all the (perennial) species from one individual to another. Frequently it develops up to the end of September as a strong, straight, little or not at all branching taproot (excepting the fibrous roots); sturdy lateral roots are often developed immediately below the cotyledons or even farther down. It is significant that in many individuals the primary root is bent or

[1] Babcock, E. B. Supplementary notes on *Crepis*. II. Phylogeny, distribution and Matthew's Principle. Evolution, **3** (4): 374–376, 1949.

[2] Mattfeld, J. Review of Babcock, E. B., The genus *Crepis* (Univ. Calif. Publ. Bot., Vols. **21**, **22**, 1947), Bot. Jahrb., Vol. **75**, Heft. 1, 1950.

knee-shaped in one or several places or has grown with cork-screw-like windings; or again that its shorter, thicker part changes suddenly (as a consequence of lesions or of a natural dying off?) into a very much thinner but longer root. This behavior certainly indicates already a weakening of the primary root. In this respect the rhizomatous and taprooted species appear to behave alike. In contrast to the annual species, these perennial species early develop adventitious roots from the base of the epicotyl. At the end of June (sown in May) *C. pontana, pannonica* and *Jacquini,* as well as always a few individuals of the other (perennial) species had not developed any adventitious roots. By the end of September these are still lacking only in *C. pannonica* and a few specimens of *C. pontana;* but they may already be present in other individuals of the latter species. The adventitious roots are strong and wiry. By the end of July they are thinner and for the most part also shorter than the main root. By the end of September, though thinner, they are for the most part much longer, but seldom at the same time also stronger, than the main root. Like the latter, they also develop fibrous roots. *Hence all species possess in their first period of vegetation a main root growing as a taproot; and when later this is missing in the rhizomatous species, it has died out at the earliest in the second year after germination, and was replaced by the adventitious roots. Generally speaking, the restriction of the root system to the adventitious roots must be considered as a derivative state* [italics mine—E. B. B.].

"In the heyday of speculative pedigree-phylogeny one would surely have applied the "law of irreversibility" to this relationship and would have said: the groups with a rhizome stem from those with a taproot. This "law," however, can no longer be applied today and especially in this case. Even according to the scanty observations just cited, the relation between adventitious and main roots is not merely an alternative between two extremes, but a labile correlation with several alternatives. It is perfectly conceivable that the development of the life span and of the strength of the primary root as well as of the adventitious roots goes in both directions. Then each of the two forms of growth could be primitive in one case and derivative in the other. When we consider, for example, that the receptacular paleae, which have disappeared in *C. capillaris* in the course of phylogenetic development, can reappear through a simple gene mutation, then the possibility cannot be simply discounted that the life span, in every case where a primary root still exists, also can be prolonged by mutations (on this alone depend the distinctions between rhizome and taproot). Hence, development may proceed not only from taproot to rhizome, but also from rhizome to taproot.

"The differences between rhizome and taproot are probably not as large as these words seem to imply. The exact morphological structure has as yet been little investigated. Only (when this structure is better known) could it be stated with certainty whether Babcock's assumption is right [referring to the original assumption on which the sequence of sections in the monograph was based—E. B. B.] that *Crepis* has arrived at the stage where the development has gone only from rhizome to taproot. However, it remains uncertain what influence the decision of this question either way would have on the system of the genus *Crepis* as established by Babcock. The influence would probably be negligible, because most of the sections containing rhizomatous species are primitive in their other characters, and because the more primitive among the sections with taprooted species already have a rather basal position. . . ."

Mattfeld's summary of his observations on the developing roots of perennial species of *Crepis* (italicized above) support the conclusions reached by the writer from his studies[3] on four species: *C. hierosolymitana* (rhizomatous), *C. Reuteriana* subsp. *Eigiana* (modified rhizomatous), *C. scaposa* subsp. *taraxaciformis* (taprooted perennial) and *C. pulchra* (annual) which were: that the taproot must be considered a more primitive structure than the rhizome; and that the rhizome is a modified caudex with adventitious roots. As has been explained before, this evidence came to hand too late to influence the sequence of the primitive sections in my monograph. For this reason the new arrangement, mentioned above, was proposed.[1] Mattfeld's opinion that no very extensive changes would be necessary is also in agreement with the fact that only one important alteration is proposed, namely, changing Section VI to Section I.

Mattfeld's acceptance of the principle involved in my conception that certain *Crepis* sections containing taprooted species were derived from rhizomatous ancestors, through gene mutations leading to adaptation to a drier environment, is especially gratifying. Conversely, as he points out, it is just as probable that all of the rhizomatous species of *Crepis* evolved from taprooted ancestors.

All in all, I am deeply impressed by the open-minded approach of Dr. Mattfeld in his thoroughly critical review of my work and I take this opportunity to express my appreciation.

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[3] Babcock, E. B. The Genus *Crepis.* Part I. Univ. Calif. Publ. Bot., **21**: 159–160, 1947.

THE STUDY OF SUBSPECIFIC ADVANCE IN THE QUATERNARY

DIRK A. HOOIJER

It is a well-known fact that among the species which were living in the Pleistocene and which are now extinct many were of comparatively large size. Phyletic size increase is a very important evolutionary trend in vertebrates as well as in invertebrates (as recently so excellently illustrated in this serial). Experience shows that large size, preferably called gigantism, is the herald of extinction in a great many groups of animals, and as such the phenomenon of increase in size in a group of animals beyond that "normal" for the group has been given much attention.

In this place attention might be drawn to the fact that Pleistocene as well as pre- and protohistoric remains referable to living species usually are larger, on the average, than the corresponding recent materials. In this case we might as well speak of a sort of general evolutionary trend that is going on in the course of the Quaternary. Species that have left descendants of their own to the present day usually were larger than they are now.

This trend toward decreasing size is, in a way, a reversal of the trend toward larger size displayed in the Tertiary Mammals (Depéret's "Law"). I do not mean to say that the Quaternary trend is comparable in scope and importance to the progressive size increase of Tertiary times, but nevertheless the trend might deserve attention of paleontologists and other students of evolution.

In current literature on Quaternary faunas, both in the Old and in the New World, we can find quite a few scattered notes as to the large average size of a given fossil or subfossil relative to recent material of the same species used for comparison. It is, however, only during the last few years that statements as to this Quaternary size decrease apparently representing a general evolutionary trend appear in the literature. It is considered as probable by some authorities that this decrease in general size is to be correlated with the warming-up of the worlds' climates since the Ice Age, which would be the working of Bergmann's Principle in a temporal rather than a geographic way.

This newly discovered rule is not without exceptions, and if exceptions are proof of anything at all they might indicate that the general decrease in size in the course of time is not controlled by environmental factors exclusively. Here, then, we would seem to see those internal forces at work which are so easy to refer to but so exceedingly difficult to understand. The only way for paleontologists eventually to grasp something of the true nature of this phenomenon lies in the collecting of more and more instances in which the phenomenon is shown.

It is really a very complex and many-sided problem to which the attention thus far has not yet sufficiently been drawn. Here are a few pertinent questions:

Is the phenomenon restricted to homoiothermal animals to which Bergmann's Rule exclusively applies? Evidently not; fishes and reptiles can be cited in evidence.

Is the phenomenon of greater magnitude in areas near retreating ice caps and glaciers than it is in tropical climatic zones? It does not seem to be so.

What is the relation between the general size of the species and the relative size decrease? Large animals have been more available and thus better studied than small ones, and present evidence would seem to indicate that small animals (shrews, bats) did not undergo much change in size.

Are teeth more easily to be affected by the working of this phenomenon than other parts of the body? Many fossil animals are known exclusively by teeth.

The more we know about the whole of an animal's morphology the more complicated becomes the picture. As a matter of fact the size decrease can never be proportionally the same for all parts of the body or for all dimensions; a harmonic decrease in size for the whole animal is a necessity for survival. The Golden Eagle of the Pleistocene of California had relatively longer wings and shorter legs than the living one, and the fossil eagle had a relatively heavier beak too. The California Condor from the Pleistocene differs from the recent in certain skull proportions. The big Pleistocene tiger from China had relatively thicker metapodials than the living one; the Pleistocene Javan rhinoceros was not only larger than the living form but also was different in the ratios of its limb segments; the subfossil Sumatran orang-utan has size proportions throughout its tooth series different from those in the living orang-utan, and so has the Moor Macaque of prehistoric Celebes. Are these all merely adaptations? Adaptations to what?

In these and many similar cases there can be no doubt that the subfossil or fossil animals are con-specific with the recent, and that we are consequently dealing with subspecific advances only. No extinction nor even migration is involved; it is evolution *in situ*. The means and modes of the various metrical characters are shifting in the course of time: we have temporal clines, chronoclines. These studies of the mi-

nute changes within one and the same species during a relatively short time-span naturally lead to problems of nomenclature, but these probably can be solved as logically as possible with our static classification system by naming subspecifically only the beginning and the end of each of the continuous clines, and regarding all the points on the cline as intermediates.

Unfortunately the nature of the material available to the paleontologist usually does not permit these intensive studies of clinal variation. The purpose of the present note is to urge those paleontologists who are in a position to handle ample collections of Pleistocene, pre- or protohistoric remains of living species to study this valuable material as intensively as possible, with the help of adequate series of recent material and all sorts of statistical methods. The study of variation is a basic task for the paleontologist.

References

Colbert, E. H. 1949. Some paleontological principles significant in human evolution. Studies in Physical Anthropology, no. 1, Early Man in the Far East, pp. 103–148 (especially pp. 127–128).

Hooijer, D. A. 1949. Mammalian evolution in the Quaternary of southern and eastern Asia. Comptes Rendus XIII^{e} Congrès Int. de Zool. Paris (1948), pp. 507–511; Evolution, vol. 3, pp. 125–128.

——. 1950. Man and other mammals from Toalian sites in south-western Celebes. Verh. Kon. Ned. Akad. v. Wetenschappen Amsterdam, afd. Natuurk., sect. 2, vol. 46, no. 2, 164 pp., 3 pls. (especially pp. 10 and 147–148).

Howard, H. 1950. Fossil evidence of avian evolution. The Ibis, vol. 92, pp. 1–21 (especially p. 19).

Newell, N. D. 1949. Phyletic size increase, an important trend illustrated by fossil invertebrates. Evolution, vol. 3, pp. 103–124, 6 figs.

Romer, A. S. 1949. Time series and trends in animal evolution, in: G. L. Jepsen, E. Mayr, and G. G. Simpson (editors), Genetics, Paleontology, and Evolution. Princeton, pp. 103–120, 2 figs. (especially pp. 111–112).

Simpson, G. G. 1949. The meaning of evolution. A study of the history of life and of its significance for man. New Haven and London, xv + 364 pp., 38 figs. (see especially p. 136).

Weidenreich, F. 1946. Apes, Giants, and Man. Chicago, 122 pp., 90 figs. (see especially pp. 47–66, and also Am. J. Phys. Anthrop., new series, vol. 7, 1949, pp. 513–518).

GEOGRAPHIC RACES AND SPECIATION

J. T. Nichols

The facts here presented are not new, but known and more or less generally accepted. It is my purpose to call attention to a possible, I believe in some cases probable, interpretation of them, commonly overlooked.

We have a classic hypothesis that (geographic) isolation plus varitaion in environment are the basic factors in speciation. Isolation of its parts (at least by distance) over an ununiform environment is rather obviously the principal factor that has divided many species into recognizable geographic races. I am speaking of vertebrates, having little knowledge of invertebrate phyla. A great many, perhaps the majority of naturalists today, are what might be called "isolationists." As such their attention is focussed on geographic races as incipient species. I do not question for a moment that species have arisen that way, and believe the proportion of species that has done so, depends somewhat on one's species concept.

We now come to a phenomenon, not so rare but that many of us are familiar with it, of a species separable into intergrading geographic races over a considerable range, but with two contrasted races in some one area, which act as species, do not mix. There is a rational isolationistic explanation for this. We may suppose that a circuitous distribution has brought the end races, those longest separated by time, and, or distance along the line they have followed, together by chance.

There is, however, an alternative hypothesis to be considered. "Two species" may have evolved and diverged by competition, at a dispersal center of abundance, where they occur, intermediate "races" elsewhere approximate their parent form, distributed from this center meanwhile. In such a case the (comprehensive) species would be expected to be more abundant there than elsewhere.

VISIBLE PLASMAGENES [1]

ERNST CASPARI

Cytoplasmic inheritance has been demonstrated in a number of instances. In some of these cases, evidence has been obtained that particulate selfreproducing entities, so-called plasmagenes, are transmitted by the cytoplasm. Since in most cases these particles are submicroscopic in size, information concerning their role in biology and development is largely indirect. Therefore it becomes important to study self-reproducing cytoplasmic structures which are large enough to be seen under the microscope. This problem has been attacked by A. Lwoff in a fascinating, thought-provoking book. The structures in question are the kinetosomes of the Ciliates, the basal granules from which the cilia originate.

The approach of the work is purely morphological. The behavior of the kinetosomes is studied in different stages of the life cycle of a particular species, and in a comparative way in different species. Experimental material, quoted from the literature, is used frequently, but is usually not necessary for the argument. Biochemical speculations are abundant, but their factual basis appears tenuous. The main value of the book is contained in the clear and convincing integration of morphological and developmental observations.

Kinetosomes are selfreproducing particles in so far as they are seen to multiply by division, and never are observed to arise *de novo*. They have a number of developmental potencies, among them the ability to form cilia, trichocysts and trichites. Trichocysts and trichites are formed at definite stages of the life cycle of the ciliate. At these stages each kinetosome may divide into a kinetosome and a trichocystosome which later will give rise to a trichocyst. Differentiated trichocysts and trichites have lost their ability to divide.

Kinetosomes are arranged on the surface of the ciliate in definite rows, so-called kineties. At the right side of each kinety is situated a fibrous structure, called kinetodesma. The kineties have a certain degree of self-reproducibility of their own: at each division, the kineties of the daughter cells are derived from the anterior and posterior parts of the corresponding kinety of the mother cell. Kineties can increase in length by division of the constituting kinetosomes, or become shortened by loss of kinetosomes. New kineties may be formed in individual development by division of the kinetosomes of a neighboring kinety. In other cases, the kinetosomes of a particular kinety start dividing and lose their orderly arrangement in the process, giving rise to an "anarchic field." The kinetosomes of an anarchic field can later become arranged in definite kineties. Since anarchic fields of kinetosomes lack kinetodesmas, while kinetodesmas become visible as soon as the orderly arrangement of the kinetosomes takes place, it is concluded that the kinetodesma is responsible for the arrangement of kinetosomes in kineties.

The kinetosomes and kineties undergo definite cyclical changes during the life cycle of the individual, and different kineties of the same individual behave differently in this respect. These changes in time and differences in space must be due to changing conditions of the underlying cytoplasm. It is argued that the underlying cytoplasmic structure is situated in the cortex, since there exists good evidence for its rigidity, a criterion not met by the freely flowing endoplasm. At least part of the cortical conditions seems to reside in the visible cortical network. In some cases, organization of the cortical network precedes organization of the kineties. At division, the cortical network is sometimes completely reorganized, and in this case, groups of kinetosomes may form centers of orientation.

In this way, the life cycle, cell division and differentiation of ciliates can be understood as resulting from the interaction of three types of cytoplasmic structures which are closely connected with each other: kinetosomes, kinetodesmas and cortical network.

These results are discussed on a chemical basis in terms of Paul Weiss's theory of "molecular ecology." It appears doubtful whether for the discussion of the behavior of kinetosomes much is gained by the use of this frame of reference. It is certain that morphological changes of the kind observed must have a physicochemical basis. But this does not mean that these morphological processes permit us to postulate the existence of specific substances such as cilia-forming and trichocyst-forming substances, or of specific building blocks for kinetosomes of different kinds.

The comparison of the life cycle of differentiation, dedifferentiation and division in ciliates with the development of metazoans is highly stimulating. Analogies certainly exist, particularly in the appearance of morphogenetic fields and in the importance of the cortex. This is particularly striking when the fundamental dif-

[1] Lwoff, André. Problems of morphogenesis of ciliates. The kinetosomes in development, reproduction and evolution. IX + 103 pp. John Wiley & Sons, Inc., 1950.

ference between these two groups of organisms is kept in mind. The ciliate remains a single cell, and all its complicated structures are formations of this cell. Most or all organels of the ciliate cell seem to be derived from kinetosomes. The life cycle of a ciliate, consisting of differentiation and dedifferentiation of organels, is therefore to a large degree dependent on the life cycle of the kinetosomes and kineties. The dedifferentiation at division is associated with a phenomenon reminiscent of the biogenetic law in metazoans; at division, ciliates frequently assume a phylogenetically more primitive organization than that of the mature "adult."

The question whether all kinetosomes of an organism are genetically identical, or whether their differences may be due to a process analogous to mutation, is discussed but not definitely decided. The question of the possible interaction of the nuclear gene system with the kinetosome system is not mentioned. That may be due to the fact that except for the experiments of Sonneborn and Beale no experimental material bearing on this problem is available. Phylogenetic considerations may, however, be used as a guiding principle, if it is admitted that evolution is mainly a genetic phenomenon. There is no evidence, at least from the material contained in the present book, that the kinetosomes of different species are different; they appear to possess the same developmental potencies in a number of widely different species. The kineties are remarkably constant in evolution. In groups of related species they can be homologized easily, and seem to serve similar functions. Their situation on the organism is, however, very variable, and the kineties appear considerably shifted from one species to another. It appears therefore that the situation of a kinety in the adult is easily influenced by genes. The number of kineties remains relatively constant in evolution, but varies between different stages of the life cycle. A systematic study of the evolution of kineties may be expected to throw much light on the phylogenetic relations in ciliates.

The book is well written and beautifully illustrated. The pleasant appearance of the book will contribute to the enjoyment the reader will obtain from the interesting facts and the original arguments and discussions presented by the author.

ISOLATION, DISPERSAL AND EVOLUTION [1]

ERNST MAYR

With isolation now recognized as one of the major evolutionary mechanisms, it is desirable that the effects and mechanisms of isolation be further analyzed. In the forests of the Indian Peninsula, particularly in the more or less isolated mountains, elements of the Malayan fauna occur, which are now widely separated from the Malayan fauna which reaches through Burma north to Assam and in part to the Himalayan slopes. How this fauna bridged the gap has long been a zoogeographical riddle. Hora proposed in 1937 the ingenious theory that the path of dispersal consisted of the Vindhya-Satpura hill ranges which connect the Western Ghats with the mountains of Assam. To have served as such an immigration pathway Hora believes that the Satpura must have been uninterrupted (there is now a large gap north of Calcutta), also higher and thus more humid than they now are, and covered with abundant forest.

Some of the various contributors to the symposium produce further facts on discontinuous ranges of Malayan elements, others discuss geology and paleoclimatology. No final picture emerges. The geological evidence is ambiguous and seems to indicate a greater age for the Garo-Rajmahal gap than postulated by Hora, although some geologists place it in the Pleistocene. Full agreement, however, exists in regard to the fact that during the Pleistocene the temperature was lower and the rainfall along these hills ranged much higher than at present. The zoogeographical importance of these hills is thus clearly established, but whether or not complete continuity existed or was even necessary remains to be determined. Throughout the symposium little attention is paid to the possibility that animals can "jump" across considerable stretches of unsuitable terrain. The members of the Malayan fauna isolated on the Indian Peninsula indicate various levels of evolutionary divergence, ranging from endemic species to subspecific identity. A more complete analysis might shed further light on the period of immigration (see also Ripley, *Evolution*, 1948).

The value of studies such as these initiated by Hora is manifold. They lead to a synthesis and meaningful interpretation of many scattered faunistic and taxonomic data; they encourage collaboration between sciences—meteorology, geology, biology—and they focus attention on unsolved problems. The study of dispersal facilities is such a problem.

[1] S. L. Hora *et al.*, 1949. Symposium on Satpura hypothesis of the distribution of the Malayan fauna and flora to peninsular India. Proc. Nat. Inst. Sci. India, **15**: 309–422.

INDEX TO VOLUME IV, 1950

adaptation, in flowering plants, 196–199; in snakes, 84–86

adaptive divergence, origin, with reference to *Anopheles*, 71–77

Anderson, Edgar, 100–101

Anopheles, bellator, homunculus, ecoclimatic divergence of, 43–63; variation of density with rainfall, 46 (fig.); map of capture stations, 47 (fig.); graphs of activity cycles, 49 (figs.); vertical distribution, 52 (fig.); graph of onset of evening activity, 54 (fig.); distribution pattern, 55 (fig.); vertical distribution of biting activity and oviposition, 57 (fig.); breeding ground, 59 (fig.); distribution of spleen rates, 62 (fig.); ecotopic specialization of *homunculus* and relation to competition with *bellator*, 64–78; breeding indices and plots, 68 (fig.); vertical distribution of oviposition, 71 (fig.); ecological relations, 73 (fig.)

antennae, role of, in mating behavior of female *Drosophila*, 149–154

Apini, caste determination in, 8

arthropods, skeletal evolution of, comparison with vertebrates, 164–171

autosomal lethals, in experimental populations of *Drosophila melanogaster*, 172–174

Babcock, Ernest B., 358–359

Blair, Frank W., 253–275

Bombini, caste determination in, 8

Brito da Cunha, A., 212–235

Burla, H., 212–235

Carduus argentatus, 105 (fig.)

Caspari, Ernst, 362–363

caste determination, evolution of mechanism of, in genus *Melipona*, 7–13; origin of the genotypic mechanism of, 9–11

Centauria virgata, 105 (fig.)

competition, between two genes in *Drosophila*, 34–42

Compositae, differentiation in aggregate fruits, 106–107; evolutionary trends, in fruiting head, 103–109; parallels in other families, 108–109; reduction in numbers of members in aggregate, 107–108; structure of fruiting head, 104–106

Crepis, 358–359; *aculeata*, 105 (fig.); *aspera*, 105 (fig.)

chromosomal polymorphism, adaptive, in *Drosophila willistoni*, 212–235

Chrysolophus, 301–315

Cyprinodon, speciation, Death Valley region, 155–163; graph correlating body circumference and temperature, 161 (fig.)

Danforth, C. H., 301–315

developmental restrictions, on recombination, in *Nicotiana*, 202–211

differentiation, 155–158

dispersal, 363

Dobzhansky, Th., 212–235

Drosophila, affinis, mating behavior, 123–134; graph of copulation vs. temperature, 131 (fig.); *algonquin*, mating behavior, 123–134; graph of copulation vs. temperature, 131 (fig.); *ananassae*, influence of light on mating behavior, 316–325; *auraria*, influence of light on mating behavior, 316–325; *equinoxialis*, geographic distribution, 214 (fig.); *melanogaster*, natural selection, 34–42; graphs of competition, 38 (fig.), 40 (fig.); role of antennae in mating behavior, 149–154; autosomal lethals, 172–174; percentage lethal chromosomes in sample, 173 (fig.); influence of light on mating behavior, 316–325; *montium*, influence of light on mating behavior, 316–325; *paulistorum*, geographic distribution, 214 (fig.); *persimilis*, experimental populations, 14–33; cf. with *pseudoobscura*, 27; gene arrangements, 17 (fig.); inversions, 22 (fig.), 23 (fig.); selection for reproductive isolation, 135–148; hybrids per generation, 139 (fig.), 140 (fig.), 141 (fig.); role of antennae in mating behavior, 149–154; insemination, 152 (fig.); *pseudoobscura*, cf. with *persimilis*, 27; selection for reproductive isolation, 135–148; hybrids per generation, 139 (fig.), 140 (fig.), 141 (fig.); antennae in mating behavior, 149–154; insemination, 152 (fig.); *rufa*, influence of light on mating behavior, 316–325; *simulans*, influence of light on mating behavior, 316, 325; *takahashii*, influence of light on mating behavior, 316–325; *tropicalis*, geographical distribution, 214 (fig.); *willistoni*, adaptive chromosomal polypmorphism, 212–235; geographical distribution, 214 (fig.); inversions, 213, 216, 218, 219 (figs.)

Dunbar, Carl O., 175–176

ecoclimatic divergence, of *Anopheles bellator* and *A. homunculus*, 43–63

ecological conditions, affect on speciation rates, 159–161

ecological factors, in speciation of *Peromyscus*, 253–275; causes of geographic variation, 254; evidence for the ecological nature of the subspecies, 261; factors in speciation of *Peromyscus*, 265; course of speciation, 270

ecotopic specialization, of *Anopheles homunculus* and relation to competition with *A. bellator*, 64–78

Elias, Maxim K., 176–177

Empetrichthys, speciation, Death Valley region, 155–163

evolution, importance of mutation rate genes in, 236–252; in pheasant hybrids, 301–315; in relation to faunal distribution problems, 363; modes discernible in taxonomy of snakes, 79–86; of heterostyly in *Primula sinensis*, 349; of mechanism of caste determination in social bees, 7–13; parallel and diverging in vertebrates and arthropods, 164–167; parallelism

in Hystricomorpha, 87–98; study of in Soviet Union, 99
evolutionary, relationships among the social bees, 7–13; trends in fruiting head of Compositae, 103–109 [monospermous fruits, 103–104; aggregate fruits, 104]
family size, in titmice (*Parus*), 279–290; nesting success, 280; survival after leaving nest, 281; variation in clutch-size, 282–290
Fassett, Norman C., 332–339
fishes, speciation in *Cyprinodon* and *Empetrichthys*, Death Valley region, 155–163; experimental work, 162; paleontological evidence, 162–163
flowering plants, protection of ovules in, 179–201; adaptation, 196–197
Fritillaria and allied genera, character combinations and distribution, 1–6
generations, percentage hybrids in *Drosophila persimilis* and *D. pseudoobscura*, 139, 140, 141 (figs.); number per year affecting speciation rates, 158–162
genes, arrangements in *Drosophila persimilis*, 14–33; competition in *Drosophila*, 34–42; differences in chromosomes from various localities, 14–33; mutation rate, 236–252
genetics, of heterostyly, 340–352
geographic, races, 361; variation, 254
gopher, 353–357
gradient, altitudinal and geographic, in relative frequency of gene arrangements, *Drosophila persimilis*, 15–17
Grant, Verne, 179–201
Gregory, William K., 164–171
Hedypnois cretica, fruiting heads of three forms, 106 (fig.); gradual reduction of pappus, 108 (fig.)
heterostyly, in *Primula sinensis*, genetics of, 340–352; complexity of, 340; evolution of, 349; variation of, within the plants, 347
Hystricomorpha, distribution, reasons for, origin of South American forms, origin of characteristics, parallelism in evolution of Old World and New World forms, 87–98
Hooijer, Dirk A., 360–361
Hora, S. L., 363
Hsu, T. C., 316–325
Hubbs, Carl L., 100–101
hybridization, introgressive, 100–101
hybridizing species, of *Phytolacca*, 332–339
hybrids, in Phasianidae, plumage traits, 301–315; description of hybrids, 303; plumage characteristics of the species and their hybrids, 303–312
Ingles, Lloyd G., 353–357
inversions, 215–230
isolation, as a cause of geographic variation, 256, 266; as an evolutionary mechanism, 363; as a factor affecting speciation rates, 158; reproductive, 135–148; sexual, measurement of, 326–331
Ives, P. T., 236–252
Kerr, Warwick E., 7–13
Koopman, Karl F., 135–148
Lack, David, 279–290
lagomorphs, fundamental sex difference from other placental mammals, 99
Lapsaneae, abortion of pappus, 107 (fig.)
light, influence on mating behavior in *Drosophila*, 316–325
Lwoff, André, 362–363
Lycopersicon esculentum, pollination relations, native and foreign, 110–122; comparative rates of cross-pollination of two mutants, 113 (fig.)
Mather, Kenneth, 340–352
mating behavior, in *Drosophila affinis* and *D. algonquin*, 123–134; in *Drosophila*, influence of light, 316–325; in female *Drosophila*, role of antennae, 149–154
Mattfeld, J., 358–359
Mayr, Ernst, 149–154; 291–300; 363
Melipona, evolution of the mechanism of caste determination in, 7–13
Meliponini, caste determination in, 8–9
Merrell, David J., 326–331
Miller, Dwight D., 123–134
Miller, Robert Rush, 155–163
mutation, as a cause of geographic variation, 254; rate genes, importance in evolution, 236–252
natural selection, in laboratory populations of *Drosophila*, competition between a white-eye gene and its wild type allele, 34–42; materials and methods, 34; viability of white and wild type flies, 35; selective mating of the red versus white phenotypes, 35; competition to extinction, 37–40; for reproductive isolation, *Drosophila pseudoobscura* and *D. persimilis*, 135–148
neontologic species and genera, vs. paleontologic, 176–177
Nichols, J. T., 361
Nicotiana, developmental restrictions on recombination in, 202–211; corollas, 203 (fig.); stages in corolla development, 204 (fig.); relative growth curves of corollas, 205, 207 (figs.); diagram of measurements on corollas, 208, 209 (figs.)
Oenanthe, polymorphism, 291–300, 293 (fig.); distribution, 294 (fig.); species characters, 298
ovule protection, in flowering plants, 179–201; in bird flowers, 181–190, 180 (fig.); in bat flowers, 190–191; in beetle flowers, 191–193, 192 (fig.); in long-tongued insect flowers, 193–196
paleogeography, distribution of Hystricomorpha, 87–90
paleontologic species and genera, vs. neontologic, 176–177
parallelism, in the evolution of Old World and New World forms of the Hystricomorpha, 95–98
Parus, major, caeruleus, ater, palustris, atricapillus, cristatus, 279–290

Patten, William, 164–171
Peromyscus, ecological factors in speciation, 253–275; distribution of five species, 272 (fig.)
Petrides, George A., 99
Phasianidae, hybrids *Phasianus* x *Chrysolophus*, 301–315
Phasianus, 301–315
phylogeny, of *Crepis*, 358–359
Phytolacca, 332–339; cf. *rivinoides* and *rugosa*, 333 (fig.); character analysis, 334, 335 (fig.); graph of plants with like characters, 337 (fig.); graph of index value and elevation, 337 (fig.)
Picus flavinucha, polymorphism, 276–277; distribution map, 277 (fig.)
pigmental variations, in pocket gophers, 353–357
Pittendrigh, Colin S., 43–63; 64–78
plasmagenes, 362–363
Pleistocene, drainage of southern Great Basin and adjoining regions, 156 (fig.); shrunken remnants of Pluvial waters of SE California, 157 (fig.)
plumage traits, in pheasant hybrids, 301–315
pollination relations, native and foreign, of *Lycopersicon esculentum*, 110–122; materials and methods of study, 111–112; observations of test plots, 112–113; additional observations, 116–119
polymorphism, adaptive in *Drosophila*, 212–235; in *Oenanthe*, 291–300; polymorph characters, 292; genetics of, 296 selective value of polymorph characters in different habitats and nature of selection, 298; intrapopulation polymorphism, 296; geographical variation, 297; in woodpecker, *Picus flavinucha*, 276–277
population size, affect on speciation rates, 158
porcupines, distribution of, reasons for, parallelism in evolution of, 87–98. See Hystricomorpha
Primula sinensis, 340–352; diagrams of, 342, 344 (figs.)
protochordates, place in history of vertebrates, 169–170
recombination, developmental restrictions on, in *Nicotiana*, 202–211
Reed, E. W., 34–42
Reed, S. C., 34–42
reproductive isolation, between *Drosophila pseudoobscura* and *D. persimilis*, 135–148; materials and methods of study, 135–138; main population cage experiments, 138–143; multiple choice experiments, 143–144; mixed-cage experiments, 144–145
reticulation, concept of, 1–2; characters of *Fritillaria* used in study of, 2–6; between *Fritillaria* and other genera, 6
rhizome, in phylogeny of *Crepis*, 358–359
Rick, Charles M., 110–122
Ripley, S. Dillon, 276–277
rodents, teeth of, 92, 93 (figs.)
Sauer, Jonathan D., 332–339
Schmidt, Karl Patterson, 79–86
selection, in *Drosophila*, 34–42; for reproductive isolation, 135–148; as a cause of geographic variation, 255
selective mating, measurement of, 326–331
sex difference, between lagomorphs and other placental mammals, 99
sexual isolation, measurement, 326–331
skeletal evolution, parallel and diverging in vertebrates and arthropods, 164–171
Smith, Harold H., 202–211
snakes, modes of evolution discernible in taxonomy, 79–86; normal snake type, 79–80; evolutionary origin of the snake group, and levels of progressive evolution, 80–81; successive adaptive radiations, 82–84; evolutionary adaptation to environment, 84–86 (fig.); evolutionary diagram, 84 (fig.); phylogenetic family tree, 85 (fig.)
social bees, evolutionary relationships, 7–8
Soviet Union, study of evolution in, [review], 99–100
speciation, in *Cyprinodon* and *Empetrichthys*, 155–163; and geographic races, 361; of *Peromyscus*, 253–275
species, concept, further discussion, 175–176; paleontologic and neontologic, 176–177
Spiess, Eliot B., 14–33
Spieth, Herman T., 316–325
Stebbins, G. L., Jr., 99–100
Stresemann, Erwin, 291–300
subspecies, 261–263
subspecific advance, in Quaternary, 360–361
taproot, in phylogeny of *Crepis*, 358–359
taxonomy, of snakes, 79–86
Thomomys bottae, 353–357; graph of population composition, 356 (fig.)
titmice, family size in, 279–290
Turrill, W. B., 1–6
variation, in clutch-size in titmice, specific, individual, with age, seasonal, annual, local, regional, 279–290; studies in *Phytolacca* hybridizing species in Northeastern Colombia, 332–339; species involved, 332; collection and analysis, 333; field observations of habitat, 337; similar hybrids in other areas, 338
vertebrates, skeletal evolution comparison with arthropods, 164–171
Wallace, Bruce, 172–174
weed, genus, *Phytolacca*, 332–339
Wood, Albert E., 87–98
woodpecker, polymorphism in, 276–277
Zirkle, Conway, 99–100
Zohary, M., 103–109